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A Tale of Two Rhythms: The Emerging Roles of Oxytocin in Rhythmic Prolactin Release

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Abstract

Hormone secretion often occurs in a pulsatile manner. In this article we discuss two rhythms of in vivo prolactin release in female rats and the ongoing research that we and others have performed to understand the mechanisms underlying them. The peptide hormone oxytocin appears to play an important role in both rhythms. One rhythm occurs during the first half of pregnancy, but can also be induced in ovariectomized rats. This is characterized by a circadian pattern with two prolactin surges per day. Two methods for triggering this rhythm are discussed, each utilizing a unique physiological pathway that includes oxytocin action, presumably on pituitary lactotrophs. The second rhythm occurs during the estrous cycle and is characterized by a surge of prolactin on the afternoon of proestrus. We discuss recent findings that oxytocin is more effective at stimulating prolactin release from lactotrophs taken from animals on the afternoon of proestrus than from those of animals on the morning of diestrus 1, raising the possibility that this hormone plays a physiological role in the regulation of prolactin secretion during the estrous cycle.

Prolactin is one of the most versatile hormones and its release from pituitary lactotrophs in female rats is stimulated by suckling and mating, and also occurs on the afternoon of proestrus (1). The wide array of factors that contribute to the control of prolactin release are reviewed in (2). Suckling evokes a classic neuroendocrine response, in which prolactin release starts when the suckling begins and ends when the suckling stops. In contrast, mating evokes a prolactin response that lasts for ten days, indicating that some type of “memory” is activated by the stimulus. During pregnancy, this response is rhythmic, consisting of two prolactin surges per day, one in the morning (the *nocturnal surge*) and one in the afternoon (the *diurnal surge*). Likewise, prolactin released during the estrous cycle is rhythmic, with a surge occurring every 4–5 days, on the afternoon of proestrus. There is now evidence that the peptide hormone oxytocin is involved in both of these rhythmic behaviors. In this article we provide an overview of recent work done in our lab to determine the role that oxytocin plays in rhythmic prolactin secretion.

Rhythm 1: Circadian prolactin rhythm induced by cervical stimulation

The circadian prolactin rhythm induced by cervical stimulation received during mating occurs during the first half of pregnancy in the female rat and is characterized by two surges per day (3, 4). The released prolactin is necessary to rescue the corpus luteum and maintain its ability to secrete progesterone for ten days (1, 2). After that, progesterone secretion is sustained for the remainder of the 20–22 day pregnancy by placental lactogens (5, 6). A

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similar prolactin rhythm, lasting up to 12 days, can be induced by artificial cervical stimulaltion in both intact and ovariectomized animals, demonstrating that ovarian steroids are not necessary for triggering or maintaining the prolactin rhythm (7). However, ovarian steroids do play a role in the termination of these surges in intact animals (see (1)). While it has been known for many years that the cervical stimulation-induced prolactin rhythm involves interactions between the hypothalamus and pituitary lactotrophs (8), questions regarding the mechanism for the initiation and maintenance of this rhythm have been hard to answer, and are largely unanswered even today. Three questions immediately come to mind: (1) how does cervical stimulation trigger the memory in ovariectomized rats? (2) what is the memory? (3) what are the elements required for the production of the prolactin rhythm that is maintained by the memory?

We have found that peripheral injection of oxytocin or central injection of ovine prolactin into ovariectomized rats can start the circadian prolactin rhythm (9, 10). Motivated by these findings, we investigated whether cervical stimulation was capable of producing a prolactin rhythm when either an oxytocin receptor antagonist or a prolactin receptor antagonist was applied centrally (via intracerebroventricular infusion) during and/or after the cervical stimulation. Central infusion of the oxytocin receptor antagonist desGly-NH_2-d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT into the lateral cerebral ventricle had little or no effect on the cervical stimulation-induced rhythm (C. Helena, unpublished observation), suggesting that central actions of oxytocin are not involved in the triggering of the memory and are not part of the rhythm mechanism. (In a different strain of rats, however, a direct injection of the oxytocin receptor antagonist into the ventrolateral region of the ventromedial hypothalamus inhibited the prolactin rhythm induced by mating, rather than cervical stimulation (11).) Central infusion of the prolactin receptor antagonist S179D inhibited the rhythm while the antagonist was present, but if the prolactin receptor antagonist was present only on the day of cervical stimulation the prolactin rhythm was still produced (10). This suggests that the central action of prolactin is necessary for the production of the rhythm (the rhythm does not occur when a prolactin receptor antagonist is present), but is not required for triggering the memory (cervical stimulation induced a delayed prolactin rhythm even though central prolactin receptors were blocked at the time of stimulation). Thus, while it remains unclear how cervical stimulation triggers the memory that maintains the prolactin rhythm, these data argue against a role for central oxytocin or prolactin in triggering the memory for the rhythm.

The identity of the memory also remains elusive, although there has been recent progress. Some recent work suggests that the ventrolateral region of the ventromedial hypothalamic nucleus may be a component of the memory (11), and since parvocellular neurons send projections to the ventromedial hypothalamus (12), the paraventricular nucleus may also be involved. Whatever the identity and location of the memory, one may ask whether it acts directly on anterior pituitary lactotrophs or whether it mediates its effect on prolactin secretion through modulation of dopaminergic neuron activities in the arcuate and periventricular nuclei of the hypothalamus. Dopamine released from these neurons is known to have a strong inhibitory action on lactotrophs (13).

The elements involved in the production of the prolactin rhythm have largely been identified. Hypothalamic dopamine is known to inhibit lactotroph activity via binding to D2-type receptors (14). In contrast, prolactin from lactotrophs stimulates hypothalamic dopamine neurons (15) through specific receptors (16). Thus, prolactin indirectly inhibits its own release through activation of the dopamine neurons. In vitro studies in rat hypothalamic slices (17) and cell cultures from the mediobasal hypothalamus of the embryonic rat pup (18) have shown that prolactin application increases dopamine synthesis through phosphorylation-mediated upregulation of tyrosine hydroxylase activity. This enzyme,

which converts tyrosine into L-DOPA, is the rate-limiting enzyme in dopamine production, and these studies showed that the increase in catecholamine levels occurs within 1–2 hr of prolactin application. Prolactin also increases the mRNA level for tyrosine hydroxylase, but this occurs on a slower time scale of 4 hr (18). This slower time scale is similar to that observed in an in vivo study (19). Another in vivo study showed that dopamine neuron activity was elevated and serum prolactin levels were inhibited within an hour of peripheral injection of ovine prolactin (15). These findings suggest an increase in dopamine secretion in response to prolactin. (There was an additional effect, with increased dopamine neuron activity and decreased prolactin levels, three hours after ovine prolactin injection.) Using mathematical modeling, we have demonstrated that the mutual feedback loop between lactotrophs and dopamine neurons, with a time delay, is capable of generating a two-pulse per day prolactin rhythm (20). A key prediction of the model is that dopamine neuron activity should peak at times between the prolactin surges. Because the ratio of DOPAC (3,4-dihydroxyphenylacetic acid, a metabolite of dopamine) to dopamine is an index of dopamine neuronal activity at the synaptic terminals (21, 22), we measured this ratio in the median eminence, the intermediate lobe, and the neural lobe of cervically-stimulated and ovariectomized rats to investigate this prediction. We found that the ratio peaked at noon in both the median eminence and the neural lobe, which is between the two prolactin surges and is consistent with the model prediction (23).

Although interactions between lactotrophs and dopamine neurons appear to be crucial for the prolactin rhythm, additional factors must be involved. Modeling suggests that vasoactive intestinal polypeptide-ergic neurons in the suprachiasmatic nucleus, which innervate (24) and inhibit dopamine neurons of the arcuate nucleus early in the morning in a circadian fashion (25, 26), are responsible for setting the time of the nocturnal surge and for making the nocturnal prolactin surge larger than the diurnal surge (20). Moreover, the fact that administration of vasoactive intestinal polypeptide antisense oligonucleotides in the suprachiasmatic nucleus altered the prolactin (and oxytocin) rhythms in cervically stimulated rats (27) demonstrates an important role for this input from the suprachiasmatic nucleus. Earlier work from our lab suggested a role for oxytocin in the regulation of prolactin surges in cervically stimulated rats (28). More recently, we showed that peripheral infusion of an oxytocin receptor antagonist blocked the cervical stimulation-induced prolactin rhythm as long as this antagonist was present. However, once the oxytocin antagonist had cleared from the system two days after the end of the infusion, the prolactin rhythm appeared (23). Since the oxytocin antagonist does not cross the blood-brain barrier, this suggests that oxytocin action on the lactotrophs is a necessary element of the prolactin rhythm. These findings also indicate that peripheral oxytocin actions are not essential for the triggering of the memory, since cervical stimulation was able to trigger the memory even in the presence of a peripheral oxytocin receptor antagonist (although the prolactin rhythm was not expressed until after the oxytocin antagonist was cleared from the system).

Figure 1 illustrates our proposed model for the cervical stimulation-induced prolactin rhythm. The prolactin rhythm is mediated primarily by the mutual interactions between lactotrophs and hypothalamic dopamine neurons (solid arrowhead indicates stimulatory action, in this case with a delay of $\tau = 2$ hrs, and open arrowhead indicates an inhibitory action). Lactotroph and dopamine neuron activity oscillate out of phase, so that the prolactin level is high when dopamine neuron activity is low and vice versa. The rhythm is facilitated by the stimulatory actions of oxytocin released from neurons of the paraventricular nucleus into the neural lobe of the pituitary (29) and into the median eminence (30). This oxytocin has been shown to increase Ca^{2+} levels (27) in lactotrophs and to stimulate prolactin secretion both in vivo (9, 31, 32) and in vitro (27, 33–35). The prolactin, in turn, has a rapid inhibitory action on oxytocin neurons of the supraoptic nucleus (36, 37) and the paraventricular nucleus (Sirzen-Zelenskaya et al., in preparation). Both of these actions are

included in the model. The circadian inhibitory action of vasoactive intestinal polypeptide neurons of the suprachiasmatic nucleus onto dopamine neurons is also included. Finally, cervical stimulation activates a memory that maintains the prolactin rhythm. It does this by partially inhibiting the dopamine neurons and by stimulating oxytocin neurons. Evidence for the latter comes from experimental data, showing that peripheral oxytocin levels are elevated following cervical stimulation (9, 38). Evidence for an inhibitory effect of the memory on secretion from dopamine neurons comes from a mathematical modeling study (20). Here we demonstrated that a direct stimulatory input to lactotrophs would lead to an elevated prolactin level, but would not initiate a rhythm. In contrast, partial inhibition of dopamine neurons both increased the mean prolactin level and, most importantly, induced a circadian prolactin rhythm. Thus, while we are still far from identifying the central location of “the memory”, we believe that progress has been made in understanding the mechanism through which it works.

When the descriptive model shown in Fig. 1 is translated into mathematical equations (20, 39) a simulated prolactin rhythm is produced (Fig. 2A, solid). In this simulation, the prolactin level is low prior to cervical stimulation. A rhythm is started by the stimulus, with two prolactin surges per day. The second, (diurnal) surge is smaller than the first, as is typically observed experimentally (23, 27). Along with the prolactin rhythm, there are rhythms in the activities of dopamine neurons (Fig. 2A, dashed) and oxytocin neurons (Fig. 2B). These variables oscillate out of phase with the prolactin rhythm, peaking when the prolactin level is declining (in the case of dopamine) or at its nadir (in the case of oxytocin). This rhythm persists as long as the memory is on (Fig. 2C).

Rhythm 1 (continued): Circadian prolactin rhythm induced by peripheral oxytocin injection

It has been shown that oxytocin is released into the circulation following cervical stimulation in sheep (40), rats (38), and humans (41, 42). This motivated us to inject oxytocin peripherally into female ovariectomized rats to attempt to start the circadian prolactin rhythm. We found that oxytocin injection starts a prolactin rhythm that is very similar to that induced by cervical stimulation (9). That is, there are two prolactin surges per day, one in the morning and another in the afternoon, which continue for at least 7 days. Do cervical stimulation and peripheral oxytocin injection trigger the prolactin rhythm in the same way? To investigate this, we performed a series of experiments with oxytocin and prolactin receptor antagonists. The results of these experiments are illustrated here (Fig. 3) with the mathematical model (for equations, see Helena et al., in preparation).

When the oxytocin receptor antagonist is administered peripherally during the cervical stimulation, the prolactin rhythm is initially inhibited. However, once the oxytocin receptor antagonist clears the system a prolactin rhythm emerges (Fig. 3A). This was demonstrated in (23); the prolactin rhythm emerged two days after cessation of the oxytocin receptor antagonist infusion. The explanation for this is that oxytocin stimulatory action on the lactotrophs is necessary for the rhythm to occur, but the trigger for the memory does not require peripheral actions of oxytocin. In contrast, when oxytocin is injected peripherally in the presence of a peripheral oxytocin receptor antagonist there is little or no effect on prolactin levels (Fig. 3B), even after the oxytocin receptor antagonist has cleared the system (Helena et al., in preparation). This shows that the peripheral injection of oxytocin triggers the prolactin rhythm by acting peripherally, most likely on lactotrophs. This is in contrast to the way that cervical stimulation triggers the rhythm, which does not require peripheral oxytocin actions.

How can peripheral oxytocin actions trigger the memory? One possibility is that the peripheral oxytocin injection stimulates lactotrophs sufficiently so that the dopamine inhibition is overcome and prolactin is released into the blood. The prolactin then crosses the blood-brain barrier, possibly through receptors in the choroid plexus (43, 44), and triggers the memory. (In fact, peripheral injection of ovine prolactin at high concentration initiates the prolactin rhythm, bypassing oxytocin altogether (10).) A model simulation illustrating the critical role played by central prolactin is shown in Fig. 3D. In this figure, a central prolactin receptor antagonist (cPRLa) is simulated. When peripheral oxytocin injection is simulated in the model there is a single large surge of prolactin, due to the direct effect of oxytocin on lactotrophs. However, when a central prolactin receptor antagonist is present at the time of the oxytocin injection no rhythm is initiated, even after the prolactin receptor antagonist is removed. This reflects the model hypothesis, supported by experimental data (10), that central prolactin can trigger the memory (Fig. 1). When this link is impaired, by central administration of a prolactin receptor antagonist, the prolactin released in response to the peripheral oxytocin injection cannot trigger the memory, so even after the prolactin receptor antagonist is cleared there will be no prolactin rhythm. This model prediction, that peripheral oxytocin injection fails to initiate the prolactin rhythm when the prolactin receptor antagonist is infused centrally on the day of the oxytocin injection, has been experimentally confirmed in our lab (Helena et al., in preparation).

This result differs significantly from a similar experiment conducted on cervically stimulated rats (without oxytocin injection). Here, the cervical stimulation-induced prolactin rhythm was prevented when the central prolactin receptor antagonist infusion was maintained continuously. However, when it was infused only during the day of cervical stimulation, the prolactin rhythm started two days later (10). This suggests that cervical stimulation triggers the memory through a mechanism that does not utilize central actions of prolactin. This is illustrated with the model in Fig. 3C. The cervical stimulation triggers the memory, thereby partially inhibiting dopamine neurons and stimulating oxytocin neurons. This results in an elevation of peripheral prolactin. However, while the central prolactin receptor antagonist is present, the positive feedback of prolactin onto the dopamine neurons is blocked, so the rhythm mechanism is dysfunctional. Once the prolactin receptor antagonist is cleared and the prolactin feedback onto dopamine neurons is restored, a prolactin rhythm is produced, since now all the required elements (triggered memory and a functional rhythm generating mechanism) have been reestablished.

From these experiments, it appears that although both cervical stimulation and oxytocin injection can induce a biphasic circadian prolactin rhythm, the pathways through which they operate are different. The model illustrated in Fig. 1 is our current view of the interaction pathways whereby cervical stimulation and peripheral oxytocin (or prolactin) injection produce the prolactin rhythm. Although our understanding of the “memory” and its location in the brain is yet very limited, the structure of the model is consistent with all of our recent data on cervically stimulated, ovariectomized rats (10, 23) and ovariectomized rats in which either oxytocin or prolactin is injected peripherally (9, 10)(see also Helena et al., in preparation). Furthermore, it clearly predicts that feedback interactions between pituitary lactotrophs and dopamine neurons are insufficient to account for mating-induced rhythmic prolactin release and establishes that additional interactions are required.

Rhythm 2: Prolactin rhythm induced by the ovarian cycle

While the first rhythm involving oxytocin does not require hormone steroids, the proestrous afternoon surge is likely due to the rhythmic variations in estradiol levels during the estrous cycle. In fact, in response to a brief exposure to estradiol, ovariectomized rats secrete daily surges of prolactin at times corresponding to that on proestrus (45). This is due in large part

to the decrease in the inhibitory influence of dopamine on lactotrophs mediated by the rise in estradiol during the cycle (1, 2). Besides this reduction in inhibition, the proestrous prolactin surge may be facilitated by a stage-specific increase in either the plasma level of a stimulating hormone, or in the responsiveness of lactotrophs to such a hormone, or both. It is well established that plasma oxytocin concentrations increase in response to estradiol administration (46) and it has been demonstrated previously that the concentration of oxytocin in pituitary portal blood reaches a peak on the afternoon of proestrus (47), following the peak estradiol concentrations observed in the morning. Moreover, passive immunoneutralization of endogenous oxytocin or peripheral administration of an oxytocin receptor antagonist has been both shown to inhibit the prolactin surge in the afternoon of proestrus (32, 48) as well as suckling-induced prolactin secretion (33, 49). Complementary to this, we have recently found that proestrous lactotrophs are significantly more responsive to oxytocin than those obtained from diestrus 1 (50). This constitutes a second rhythm, linked to the 4–5 day long estrous cycle of female rats.

In our experiments, anterior pituitaries were obtained from cycling rats on the morning of diestrus 1 or the afternoon of proestrus. Oxytocin was then applied to the anterior pituitary cells (100 nM for 10 min) and the prolactin response measured using radioimmunoassay. As shown in Fig. 4A, the prolactin response to oxytocin was much greater in cells harvested during proestrus than in cells harvested during diestrus 1. This effect was shown to be statistically significant when data were analyzed from 7 experiments from diestrous cells, and 10 experiments from proestrous cells (50). The enhanced prolactin secretion on proestrus corresponded to an enhanced intracellular Ca^{2+} response from lactotrophs (Fig. 4B). Indeed, both the number of lactotroph cells showing a Ca^{2+} response to oxytocin and the single-cell response were greater on proestrus than on diestrus 1 (50). This agrees with the observation that oxytocin receptors are up-regulated by estradiol in the anterior pituitary as shown by our lab (49) and others (51). In addition to receptor upregulation, it is likely that ovarian steroids have additional effects, since the dose-response curve of the prolactin-releasing effect of oxytocin was left-shifted in lactotrophs obtained from proestrous versus diestrus rats. Receptor upregulation alone would have no effect on the half-maximal location of the curve. Thus, it is possible that the enhanced oxytocin response on proestrus is due to an increase of anterior pituitary oxytocin receptor expression and signaling produced by the elevated estradiol levels present in the preovulatory phase of the estrous cycle.

These data show a rhythmic variation in the responsiveness of lactotrophs to oxytocin during the estrous cycle. The increased responsiveness on proestrus follows a common theme in oxytocin signaling, where the actions of oxytocin are typically preceded by an increase in responsiveness of the target tissue. For example, myometrial cells of the uterus are sensitized to the contractile effect of oxytocin immediately prior to parturition (52, 53). In the mammary gland, there is an increase in the number of oxytocin binding sites throughout gestation that remains during the period of lactation (54). Thus, the finding that the oxytocin action on lactotrophs is magnified at the time point when such enhancement is needed is consistent with a physiological role for oxytocin in the regulation of prolactin secretion.

Summary

As is the case with many hormones, prolactin secretion is often rhythmic. The particular rhythm depends on the physiological state of the animal, and the factors mediating one rhythm could be different from those mediating others. However, oxytocin and dopamine appear to be factors involved in most (if not all) of the rhythms. Much work remains to be done in the identification of the “memory” involved in the circadian prolactin rhythms initiated by mating, cervical stimulation or peripheral oxytocin/prolactin injection. This memory is also likely responsible for the phenomenon of “delayed pseudopregnancy” in the

rat, in which mating (55), electrical stimulation of the cervix (56), or electrical stimulation of the hypothalamus (57) early in the estrous cycle leads to a circadian prolactin rhythm only after ovulation occurs at the end of the estrous cycle. In addition to the systems-level question of the memory, important cellular questions remain. For example, the intracellular signaling pathway involved in the oxytocin stimulation of prolactin release is largely unknown, as is the mechanism through which this is magnified on proestrus. We hope that the many questions that remain will be answered in the near future.

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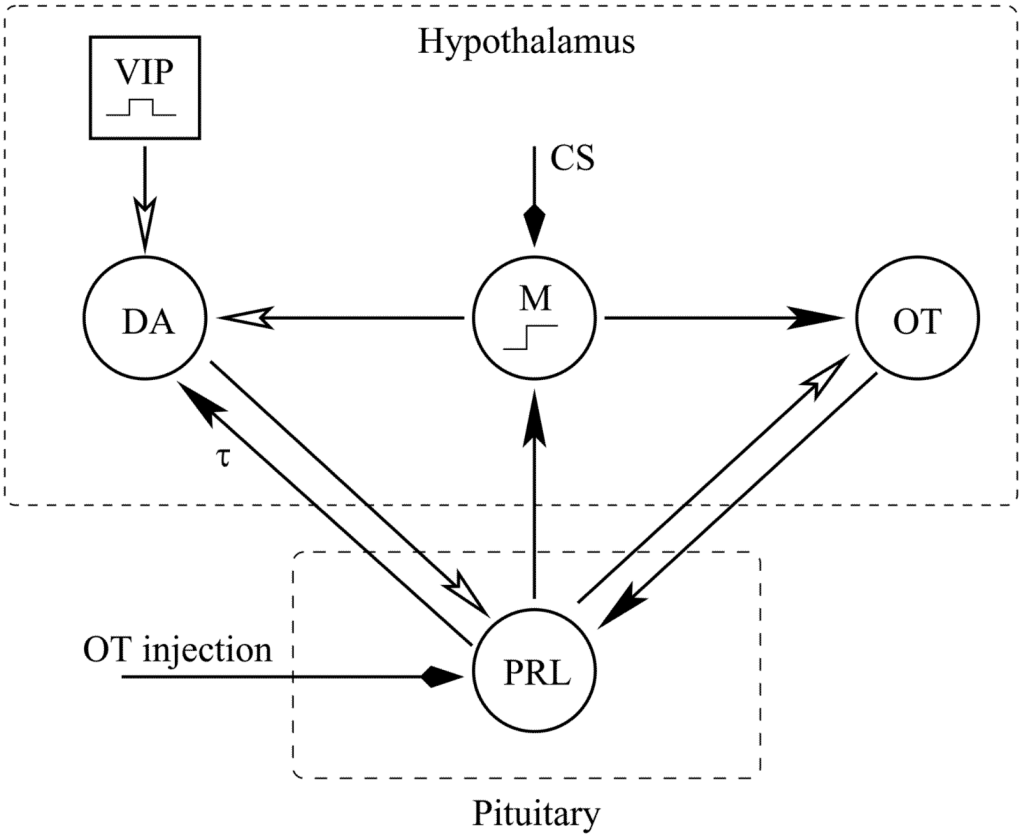

Figure 1.
Proposed model for the prolactin rhythm initiated by cervical stimulation (CS) or by oxytocin (OT) injection. Closed arrowheads indicate stimulatory actions, open arrowheads indicate inhibitory actions. The stimulation of dopamine (DA) neurons by prolactin (PRL) has a delay of τ hours. A pulse of vasoactive intestinal polypeptide (VIP) from suprachiasmatic neurons occurs every morning, setting the phase of the prolactin oscillation. The memory, M, is switched on by CS or by PRL.

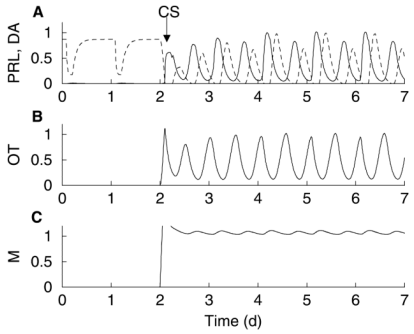

Figure 2.

Numerical simulation of the mathematical model (see (39) for equations). (**A**) A prolactin rhythm (solid) is initiated by cervical stimulation, as described in Fig. 1. The dopamine neuron activity (dashed) oscillates out of phase with prolactin levels. (**B**) The cervical stimulation activates oxytocin neurons of the paraventricular nucleus, which oscillate out of phase with prolactin levels due to the inhibitory influence of prolactin on the neurons. (**C**) The memory is switched on by the cervical stimulation and stays on throughout the simulation. The variables PRL, DA, and OT have all been normalized to facilitate comparison.

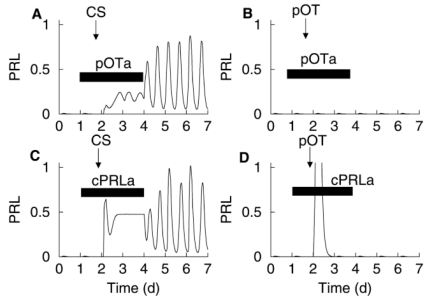

Figure 3.

Numerical simulation of the mathematical model illustrating the effects of two antagonists on the triggering of the memory for the biphasic circadian prolactin rhythm. (**A**), (**C**) Both peripheral oxytocin receptor antagonist (pOTa) and central prolactin antagonist (cPRLa) prevent the rhythm induced by cervical stimulation while the antagonist is present. However, the rhythm emerges once the antagonist has cleared from the system. (**B**), (**D**) The prolactin rhythm induced by peripheral oxytocin injection (pOT) is not triggered when the same antagonists are present during the injection.

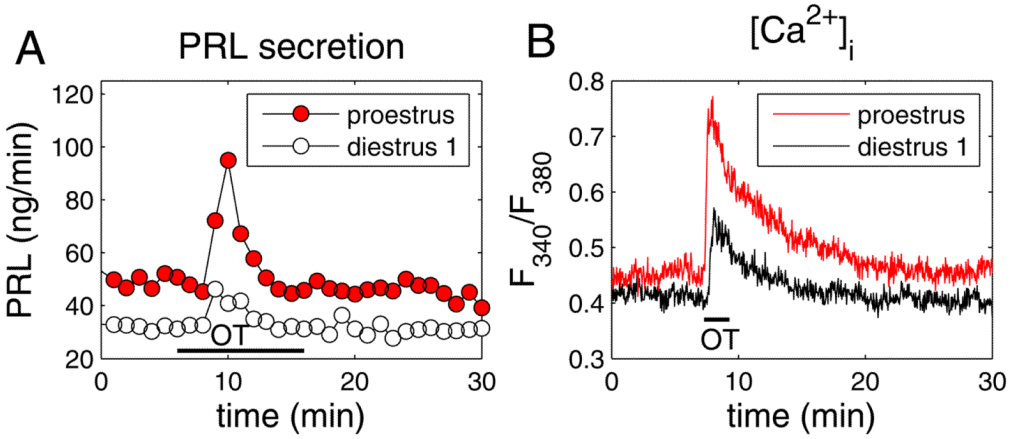

Figure 4.

Comparison of the oxytocin-induced responses in perifused lactotrophs obtained from female rats on the morning of diestrus 1 (open symbols) and the afternoon of proestrus (closed red symbols). (**A**) Oxytocin application (100 nM for 10 min) evoked a larger prolactin-releasing effect from proestrus cells. Samples were collected every minute. (**B**) Oxytocin application (100 nM for 2 min) elicited a larger increase in the free intracellular Ca^{2+} concentration, as measured by the fura-2 fluorescence ratio. See (50) for details and a full description of experimental procedures.