Effect of photoperiod on the mechanical response of the pregnant rabbit uterus to oxytocin

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MUCH EFFORT HAS BEEN DIRECTED to understanding the processes triggering parturition, and although this is still not completely understood, some common features are emerging. One is that parturition has a strong diurnal component such that mammals usually give birth during their species-typical rest period (reviewed in Refs. 14, 35, 36, 54); nocturnal mammals such as the rat during the day (28, 39, 52, 61) and diurnal mammals such as sheep (Ref. 30; own observations), monkeys (15, 16, 56), and humans (9, 10, 29, 49, 55, 64) during the night. Moreover, in humans, births occurring at night are typically of shorter duration, require fewer interventions, and have an obstetrically better outcome than births occurring during the day (Refs. 3, 9, 55, 60; reviewed in Ref. 36).

A second common feature is the importance of the hormone oxytocin (OT), released at the time of parturition from the posterior pituitary into the bloodstream, in helping to initiate and sustain the uterine contractions necessary for expulsion of the fetus(es) (1, 11, 12, 17, 19, 23–25, 31, 33, 50, 67). In those species in which it has been studied, principally macaque monkeys and humans, there is a diurnal rhythm in circulating levels of OT, with the highest levels at night (18, 20, 33, 34, 65). This rhythm becomes more pronounced as gestation progresses and is most marked at the time of parturition (Refs. 20, 33, 65; reviewed in Refs. 36, 59). It is paralleled by a diurnal rhythm in uterine activity (Refs. 32, 54, 56; reviewed in Refs. 27, 36) and in sensitivity of the myometrium to OT (5, 34), culminating in a marked increase in the expression of OT receptors at term (1, 23, 48, 62, 67). These findings, however, have been based on the study of rather few and mainly diurnal species so that information from a wider range of animals is desirable before concluding that these observations represent a general mammalian pattern.

The European rabbit (Oryctolagus cuniculus), belonging to the family Lagomorpha and thus taxonomically distinct from rodents, ruminants, and primates, is a useful addition. It is a classic laboratory mammal in the study of parturition (11, 17, 19, 22, 50, 58, 62, 63, 66) and shows marked photoperiodic organization of parturition and nursing (reviewed in Refs. 37, 41, 47).

Rabbits are predominantly nocturnal (Refs. 44, 57; reviewed in Ref. 47) and usually give birth during the early daylight hours (own observations), with parturition generally lasting no more than 10 min for the birth of up to 14 young (22, 39, 50). Nursing, however, usually occurs at night (Refs. 45, 53; reviewed in Refs. 41, 47) just once every 24 h for ~3 min (13, 38, 40, 51, 68), at which time the pups drink up to 25% of their body weight (38, 40, 51). This rapid milk transfer depends on the large and sudden release of OT into the mother’s bloodstream in response to suckling by the young (6, 12, 21, 26). Rabbits also have a marked postpartum estrus and, under natural conditions, are often both pregnant and lactating (8, 38, 51).

In a previous study (43), our laboratory found that lactating mothers, simultaneously pregnant with a second litter, refused...
or hesitated to nurse the first litter when forced to do so during the daytime, whereas nonpregnant mothers nursed without hesitation. Pregnant, simultaneously lactating females forced to nurse during the daytime also had severe birth difficulties (delayed parturition, large number of stillborn but apparently normally developed young), whereas equivalent females allowed to nurse at night had no such problems. Pregnant females injected daily with OT during the daytime to simulate daytime nursing also had subsequent birth difficulties, whereas pregnant females injected at night gave birth normally. We interpreted these findings as indicating that the pregnant rabbit uterus is particularly sensitive to OT during the daytime (the normal time of parturition) and that rabbits nurse at night to minimize the effect on the pregnant uterus of the sudden liberation of OT accompanying nursing.

These observations, together with the timing of birth in other species, led us to propose that, in the rabbit, there is a diurnal fluctuation in the sensitivity of the pregnant uterus to OT, with the time of greatest sensitivity corresponding to the normal time of parturition during the early daylight hours. It was the aim of this study to test this hypothesis by recording the responsiveness of the term-pregnant rabbit uterus to the application of OT at different times across the 24-h light-dark cycle.

METHODS

Experimental procedures were conducted according to the Guidance Principles for Research Involving Animals and Human Beings of the American Physiological Society (2) and the guidelines of the Faculty of Medicine, National University of Mexico.

Animals

Primiparous and virgin adult New Zealand white rabbits weighing 3.5–4.5 kg were used. They were bred and kept in the Faculty of Medicine animal house on a 16:8-light-dark cycle at 20–22°C, with food (Purina rabbit chow) and water available continuously, and were fed and cleaned between 0900 and 1100. The light-dark cycle was chosen to correspond approximately to natural conditions at the height of the breeding season for rabbits in Europe (42), and animals were placed 2 wk before mating in the light-dark cycle appropriate for their chosen to correspond approximately to natural conditions at the height of the breeding season for rabbits in Europe (42).

Experimental Groups

Pregnant animals. GROUP P1. These animals (n = 20) were kept on the 16:8-light-dark cycle with lights on from 0600–2200. At term (day 30–31 of gestation) a 1 × 0.5-cm longitudinal strip of myometrium was immediately taken from the middle third of one of the uterine horns and mounted in an organ bath for electromyographic recording (see Isometric Recording of Uterine Contractions). Animals were killed at four time points (n = 5/time): group P1a at 0700 (early light phase), group P1b at 1300 (midlight phase), group P1c at 2400 (early dark phase), and group P1d at 0400 (late dark phase).

GROUP P2. In this group (n = 10), the photoperiod was advanced by 12 h 2 wk before mating (lights on 1800–0000), and on day 31 of gestation uterine strips were taken as in group P1 but at two time points (n = 5/time); group P2a at 1900 (now the animals’ subjective early light phase corresponding to group P1a), and group P2d at 1400 (now the animals’ subjective dark phase, approximately corresponding to group P1d).

Estrous animals. GROUP E. These animals (n = 10) were kept and treated as for group P1. Because unmated female rabbits kept in long photoperiod remain in constant estrus (42), the females of this group were assumed to be sexually receptive at the time they were killed. They were killed at the two time points (n = 5/time) showing the greatest difference in uterine responsiveness in the P1 animals: group Ea at 0700 (early light phase), and group Ed at 0400 (late dark phase).

Isometric Recording of Uterine Contractions

The uterine strips were suspended vertically in a 6-ml organ bath and under 0.5-g light tension. The two ends of the strips were secured with fine silk threads, one end to the bottom of the chamber and the other to an isometric force transducer (FT03C, Grass Instruments) connected to a polygraph (79D, Grass Instruments). The strips were immersed in a Krebs Ringer solution [Baker; composition (in mM): 120.6 NaCl, 5.9 KCl, 1.2 MgCl2, 2.5 CaCl2, 15.5 NaHCO3, 1.2 KH2PO4, 11.5 glucose], adjusted to pH 7.4, filtered (pore size, 0.22 μm), kept at 37°C, and continuously bubbled with a mixture of 95% O2–5% CO2. The solution flowed through the chamber at 1 ml/min throughout the experiment, and 1-ml solutions of OT (Sigma) ranging in concentration from −log 1 × 10−12 to −log 1 × 10−6 M were applied by pipette to the bottom of the chamber.

Experiments started when the strips showed stable basal tension and stable rhythmic contractions. If this failed to occur within 10 min, a fresh strip from the same animal was taken. This happened in three cases, with recordings successfully obtained from the second preparation. A 30-min baseline recording was then made before OT was applied, starting with −log 1 × 10−15 M. If this produced a contractile response (as in group P1a, for example), four additional concentrations were applied in ascending order. If there was no response to the lowest dose (as in group P1b, c, and d, for example), successively higher concentrations were applied until a contractile response was obtained, after which additional concentrations continued to be applied in ascending order until the highest designated concentration of −log 1 × 10−6 M was reached. Because of the relative insensitivity of tissue taken from the P1c, P1d, P2d, Ea, and Ed animals, responses were only obtained for four or three concentrations in these groups. There was an ~15-min interval between each application of OT. This sequence was repeated four to five times for each preparation, with each experiment lasting ~5 h.

In groups P2a and P2d, two 1-ml concentrations of high-potassium Ringer solution (20 and 100 mM KCl) were administered 20 min after the last application of OT, allowing for a return to basal tension between two applications. This was done to produce a brief depolarizing challenge to check the viability of the tissue and the specificity of the effect of OT on uterine tension in relation to time of day.

Data Analysis

Changes in tension after administration of OT or KCl were quantified using an image analysis program (MICID, Image Research) to measure the area under the curve defined as the moment the pen of the polygraph was deflected upward until it returned to the basal level. This lasted for ~2–6 min (Fig. 1). The contractile response of the strips to OT is expressed both as the maximum absolute tension and using the dose-response curves for each group to determine EC50.

Descriptive values are expressed as means ± SE, and because of small sample sizes the statistical significance of differences among groups was tested using nonparametric one-way Kruskal-Wallis ANOVA followed by Dunn’s post hoc multiple comparisons. Comparisons between groups P1a and P2a and between P1d and P2d to test the effect of geographic vs. subjective time on the response of the strips to OT, and between groups P1a and Ea (0700) and between P1d and Ed (0400) to test the effect of late pregnancy compared with
estrus, were made using Mann-Whitney’s U-tests. Mann-Whitney’s tests were also used to compare values obtained after KCl was administered to groups P2a (0700) and P2d (0400), and Wilcoxon’s tests were used to compare within-group responses to the two doses of KCl. All tests were two-tailed, and an α value of 0.05 was taken as the level of significance.

RESULTS

Response of Strips from the Pregnant Uterus to OT Across the Light-Dark Cycle

Table 1 shows the maximum tension developed in response to different doses of OT by uterine strips of group P1 animals, recorded at four times during the 24-h day, and Fig. 1 shows examples of the maximum response recorded at each of the time points. Strips from group P1a (0700) were clearly the most sensitive to OT, followed by strips from group P1b (1300), from P1c (2400), and finally from P1d (0400). A Kruskal-Wallis ANOVA showed the difference among the groups to be highly significant (H = 59.2, degrees of freedom = 3, P < 0.0001). As shown by post hoc Dunn comparisons, this was mainly due to the large, ~200-fold increase in myometrial responsiveness to OT for P1a compared with P1c and P1d (P < 0.0001), although the values for P1b also differed significantly from P1a and P1c (P < 0.05) and even more strongly from P1d (P < 0.01).

To better visualize the differences in sensitivity of uterine tissue taken at the four time points and to calculate the differences in EC50, responses to OT were also expressed as relative tension (Fig. 2). Again, strips from the P1a animals (0700) were, by three to four orders of magnitude, the most sensitive; strips from the P1d animals (0400) were the least sensitive; and strips from the P1b (1300) and P1c animals (2400) were intermediate. These differences were highly significant (H = 18, degrees of freedom = 3, P < 0.0005), with the greatest difference (Dunn’s post hoc tests) between P1a vs. P1c, P1d (P < 0.0001), and P1b vs. P1d (P < 0.01), although

Table 1. Tension shown by strips from the longitudinal layer of the pregnant rabbit uterus in vitro after OT application at different times of the light/dark cycle

<table>
<thead>
<tr>
<th>Group</th>
<th>Hour</th>
<th>log OT, M</th>
<th>Tension, mN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td>12</td>
<td>88±5.5</td>
<td>119±15</td>
</tr>
<tr>
<td>P1a</td>
<td>0700</td>
<td>342±21</td>
<td>490±15</td>
</tr>
<tr>
<td>P1b</td>
<td>1300</td>
<td>600±20</td>
<td>10±2.5</td>
</tr>
<tr>
<td>P1c</td>
<td>2400</td>
<td>50±3.0</td>
<td>80±2.5</td>
</tr>
<tr>
<td>P1d</td>
<td>0400</td>
<td>111±3.0</td>
<td>130±3.0</td>
</tr>
</tbody>
</table>

Dark

<table>
<thead>
<tr>
<th>Group</th>
<th>Hour</th>
<th>log OT, M</th>
<th>Tension, mN</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1c</td>
<td>2400</td>
<td>6±0.5</td>
<td>12±1.0</td>
</tr>
<tr>
<td>P1d</td>
<td>0400</td>
<td>20±1.0</td>
<td>32±0.5</td>
</tr>
<tr>
<td>P1a</td>
<td>0700</td>
<td>14±1.0</td>
<td>26±1.0</td>
</tr>
</tbody>
</table>

Data are means ± SE. OT, oxytocin; P1a–P1d, term-pregnant animals measured at 0700, 1300, 2400, and 0400, respectively.
P1b vs. P1a, P1c also differed significantly ($P < 0.05$). Furthermore, the difference in maximal tension developed by strips from the different groups was more strongly related to the light-dark cycle than to the exact length of pregnancy at term (Fig. 3). Thus strips from group P1a (0700), which showed the strongest response to OT, were taken from animals 739 h after mating, whereas strips from groups P1b (1300), P1c (2400), and P1d (0400), listed in decreasing order of sensitivity to OT, were taken from animals 745, 756, and 735 h after mating, respectively ($H = 52.9$, degrees of freedom $= 3$, $P < 0.0001$; Dunn: P1a vs. P1c and P1d ($P < 0.0001$), although the values for P1b also differed significantly from P1a and P1c ($P < 0.05$), and even more strongly from P1d ($P < 0.01$)).

These results suggest that the strips obtained in the light phase, and particularly the early light phase, were more sensitive to OT and developed greater tension in response to its administration than strips obtained in the dark phase and that this was not due to the exact length of gestation in these term-pregnant females at the time of testing.

**Effect of Advancing the Light-Dark Cycle on the Response to OT of Strips From the Pregnant Uterus**

When, in group P2, photoperiod was advanced by 12 h, the uterine strips showed an increase in tension in response to the administration of OT similar to the response of strips from the P1 animals tested at the corresponding geographic times of the light-dark cycle (Fig. 4). Expressed as $EC_{50}$, strips from group P2a, with a 12-h-shifted subjective time of day corresponding to group P1a (0700), showed a similarly high sensitivity to the administration of OT, whereas group P2d, with a subjective time corresponding approximately to group P1d (0400), showed a similarly low sensitivity to the administration of OT. Differences among the four groups were highly significant ($H = 15.8$, degrees of freedom $= 3$, $P < 0.001$). Post hoc tests showed this to be principally due to the difference between P1a vs. P1d ($P < 0.001$) and between P2a vs. P2d ($P < 0.01$). There was no significant difference between the values for P1a vs. P2a or between P1d vs. P2d.

Together, these results suggest that, also on the inverted light-dark cycle, sensitivity of the pregnant uterus to OT was greater during the light than during the dark phase and thus was dependent on the subjective rather than on the geographic phase of the 24-h day.

**Response of Strips From the Uterus at Estrus to OT in the Light and in the Dark Phase**

Uterine strips taken from the estrous animals of group E showed an increase in tension in response to OT in a dose-dependent manner qualitatively similar to the pregnant animals of group P1 (Fig. 5). Expressed as $EC_{50}$, strips from groups P1a and Ea (0700) showed higher sensitivity to OT for their respective conditions than strips from groups P1d and Ed. Differences among groups were highly significant ($H = 14.7$, degrees of freedom $= 3$, $P < 0.002$; Dunn: $P < 0.001$ for P1a vs. Ea, P1d, Ed). However, although the pattern of response to OT of strips from the estrous animals in relation to the light-dark cycle was similar to that of the pregnant animals, it was not as strong, and the difference in the responses of strips taken from the estrous animals in the light and dark phases was not significant. Furthermore, the sensitivity of strips obtained from the pregnant animals both in the light and dark phase was greater than for the strips obtained from the estrous animals in either phase (Fig. 5).
Response of Strips from the Pregnan t Uterus to KCl in the Light and in the Dark Phase

Figure 6 shows the maximum tension developed by the strips from groups P2a (subjective time 0700) and P2d (subjective time 0400) in response to 20 and 100 mM high-potassium Ringer solution administered at the end of the test period after recording the response to OT. Whereas a significant difference in the response to the two concentrations of KCl was recorded within each group (Wilcoxon: \( T = 21, n = 5, P < 0.03; T = 21, n = 5, P < 0.03 \) for P2a and P2d, respectively), there was no significant difference in the response to the two concentrations between the two groups (Mann-Whitney: \( U = 13, n_1 = 5, n_2 = 5, P > 0.5; U = 12.5, n_1 = 5, n_2 = 5, P > 0.4 \) for 20 and 100 mM KCl, respectively).

These results suggest that the contractile response of the strips to the administration of KCl was not influenced by the stage of the high-dark cycle and thus that the photoperiod-dependent response to OT was specific and not the result of a general change in mechanical properties of the muscle.

DISCUSSION

The present findings support the prediction of a diurnal fluctuation in the sensitivity of the pregnant rabbit uterus to OT, with the time of greatest sensitivity corresponding to the normal time of parturition during the early daylight hours. The strength of contractions recorded in uterine strips in response to the application of OT was dose dependent and was \( \sim 200 \) times greater at doses three to four orders of magnitude lower in tissue taken from pregnant animals during the early light phase of the 24-h day than in tissue taken during the dark phase. Consistent with this, strips from nonpregnant estrous females also showed a tendency, albeit nonsignificant, to greater sensitivity and contractile force in response to OT when taken in the light than when taken in the dark phase. Also consistent with the effect of photoperiod on uterine sensitivity to OT, strips taken from pregnant females maintained on a light-dark cycle advanced 12 h showed significantly greater sensitivity and force in response to OT during the new subjective light than during the new subjective dark phase. And finally, the photoperiod-dependent contractile response of the uterine tissue to OT appeared specific and not simply the result of a change in general mechanical properties of the muscle since administration of KCl produced dose-dependent contractions of almost identical magnitude in strips taken in both the light and the dark phase.

The results of this study are thus consistent with previous reports that mammals usually give birth during their species-typical rest period (reviewed in Refs. 14, 35, 36, 54), that this is paralleled by a diurnal rhythm in uterine activity (Refs. 32, 54, 56; reviewed in Ref. 36) and in sensitivity to KCl produced dose-dependent contractions of almost identical magnitude in strips taken in both the light and the dark phase.

The findings are also consistent with the fact that rabbits usually give birth during the day but nurse their young at night (Refs. 41, 43, 45, 46; reviewed in Refs. 37, 47). Because under natural conditions rabbits are often both pregnant and lactating (8, 38, 51), the sudden surge in blood-born OT accompanying nursing (6, 12, 21, 26) could induce abortion or premature birth were it not timed to occur during the period of minimum uterine sensitivity to OT, that is, during the night (43). The present findings thus show a close correspondence between uterine sensitivity to OT and the previously reported diurnal partitioning of nursing and parturition in this species (Ref. 43; reviewed in Refs. 37, 47). Whether such temporal partitioning of parturition and nursing occurs in other mammals has, to our knowledge, not been investigated.

Unknown, however, are the physiological mechanisms underlying the diurnal fluctuation in sensitivity of the rabbit myometrium to OT. One likely candidate is a diurnal rhythm in

![Figure 5. Relative tension recorded in strips from the longitudinal layer of the rabbit uterus in vitro in term-pregnant (P1a, P1d) and estrous animals (Ea, Ed) in response to OT administered during the light (0700) or dark phase (0400). Comparing EC_{50}, strips obtained in the light phase both from pregnant and estrous females were significantly more sensitive to OT than strips obtained in the dark phase for either of the corresponding groups (Kruskal-Wallis ANOVA, Dunn’s post hoc comparisons). Values are means ± SE. Significant differences in EC_{50} between the indicated groups: ***P < 0.001. The values for P1a and P1d are the same as those shown in Fig. 2.]

![Figure 6. Maximum tension recorded in strips from the longitudinal layer of the term-pregnant rabbit uterus in vitro in response to 20 and 100 mM KCl administered in the light (0700) and dark phase (0400) (n = 5/group). Stips from both the light and dark phase showed significantly different dose-dependent responses (Wilcoxon), which did not differ significantly between the two phases (Mann-Whitney). Numbers inside the columns represent hours of pregnancy. Values are means ± SE. Significant differences between the indicated groups: *P < 0.05. The preparations were the same as P2a and P2d in Fig. 4.](http://ajpregu.physiology.org/DownloadedFrom)
the expression of OT receptors. Certainly, the increase in OT receptors in the myometrium preceding parturition and during labor (1, 48, 67), including in the rabbit (62), demonstrates that such rapid, short-term changes can occur. However, a study of OT receptor expression in the term-pregnant rabbit myometrium across the 24-h day is now necessary to check whether diurnal fluctuations in this underlie the diurnal pattern of uterine sensitivity to OT found here. Even if this should be the case, the question remains what, in turn, determines it. One possibility is that a diurnal fluctuation in the ratio of estradiol to progesterone (25, 29) damps OT receptor expression at night so that nursing can occur without prematurely triggering parturition, whereas enhanced expression during the daytime ensures that parturition takes place during the rabbit’s rest period. The question also remains whether such fluctuations represent endogenous circadian processes, which persist in the absence of external environmental signals. This seems likely given reports that the time of day at which birth occurs in rats (61) and primates (Ref. 4; reviewed in Ref. 35) depends on the physiological preparedness of the uterus and thus may not be obstetrically optimal.

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