Effect of photoperiod, injection of pentobarbitone sodium or lesion of the suprachiasmatic nucleus on pre-partum decrease of blood progesterone concentrations or time of birth in the rat

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Summary. In Sprague–Dawley rats kept under 14L:10D (lights on 05:00–19:00 h), parturition occurred during the light phase on Day 23, and the pre-partum decrease in progesterone concentrations was observed between 07:00 and 15:00 h during the light period on Day 22. When the rats were transferred to reversed light–dark regimen (lights on 17:00–07:00 h) on Day 7, the progesterone decrease and parturition still occurred during the light period on Day 21 and 22–23, respectively. However, when rats were kept in constant darkness from Day 7, parturition occurred independently of the time of day between Day 22 and 24. A gradual decline of progesterone concentrations was randomly observed in individual rats. In Wistar rats kept under the usual light–dark regimen, parturitions were biphasic, occurring during the light periods on Day 22 and 23. The progesterone decrease occurred at the usual time even when the lighting regimen was changed only on the day of the expected progesterone decrease. However, treatment with pentobarbitone sodium at 15:00, 19:00 or 21:00 h, but not at 12:00 or 23:00 h, on Day 21 resulted in a delay of progesterone decrease and of parturition. Complete lesion of the suprachiasmatic nucleus on Day 13 or 14 led to advancement and random distribution of the time of birth.

These results suggest that the time of parturition and of pre-partum progesterone decrease may be closely associated with an endogenous circadian system, and a luteolytic factor involving the nervous system may be present during a limited period before parturition.

Introduction

Parturition in rats generally occurs during the light period in a light–dark regimen (Mitchell & Yochim, 1970; Plaut *et al.*, 1970; Lincoln & Porter, 1976, 1979; Bosc & Nicolle, 1980; Sherwood *et al.*, 1983). This specific effect of the light on the time of parturition has been suggested in experiments involving shifts of photoperiod near mid-pregnancy (Lincoln & Porter, 1976; Sherwood *et al.*, 1983) or different light regimens, such as reversed light–dark cycles or extension of the light period each day (Mitchell & Yochim, 1970; Plaut *et al.*, 1970; Bosc & Nicolle, 1980). A link between the daily photoperiod and the time of birth may therefore be associated with an endogenous circadian rhythm of the rat (Lincoln & Doyle, 1978; Lincoln & Porter, 1979). A pre-partum increase of serum relaxin and decline of progesterone concentrations have also been suggested as being associated with the photoperiod. Sherwood *et al.* (1983) showed that the time of birth, maximal relaxin concentrations and decline of progesterone values during the pre-partum period were

advanced by alteration of the photoperiod near mid-pregnancy. As far as we know, however, there has been little study of the pre-partum decrease of blood progesterone concentrations under various lighting conditions (Bartholomeusz *et al.*, 1976; Sanyal, 1978). In our colony of Sprague-Dawley rats, parturition occurs at 09:00–15:00 h on Day 23. However, these hours of birth seem to be different from those reported for Wistar (Lincoln & Porter, 1976; Bosc & Nicolle, 1980) or Holtzman (Mitchell & Yochim, 1970) strain rats kept in the same lighting regimen. We have therefore investigated the progesterone concentration changes and time of birth in rats kept in various lighting conditions using Sprague-Dawley or Wistar rats. The effects of pentobarbitone sodium or lesions of the suprachiasmatic nucleus (herein designated SCN), which has been proposed to be the oscillator of endogenous circadian rhythm (Moore & Eichler, 1972; Inouye & Kawamura, 1979; Rusak & Groos, 1982), were also studied.

Materials and Methods

Animals. Primiparous Sprague-Dawley rats bred and reared in our laboratory and Wistar-derived rats purchased from the Animal Reproduction Company (Wistar-Imamichi strain; Chiba, Japan) were used. They were kept in a constant-temperature room ($23 \pm 1^\circ\text{C}$) under white fluorescent lighting with a light regimen of 14 h light and 10 h darkness (14L:10D; lights on at 05:00 h). They had free access to food and drinking water. At about 90 days of age, rats showing a regular 4-day cycle according to the vaginal smear cell patterns were caged with a male on the day of pro-oestrus. Successful mating was confirmed by the presence of spermatozoa in the vagina, and this day was designated as Day 1 of pregnancy. The period from Day 20 to birth was designated as the pre-partum period. The mated animals were isolated in individual cages until parturition.

Collection of samples. Blood samples were taken from individual rats at 4-h intervals from Day 20 or 21 by the tail-tip incision method (Takahashi *et al.*, 1977), with the modification described by Murakami *et al.* (1986). Blood (50 μl) was obtained by heparinized micropipette by aspiration by mouth. Each blood sample was duplicated in a volume of 20 μl and mixed with 500 μl distilled water before storage at -20°C until progesterone analysis.

Determination of time of birth. The time of occurrence of parturition was determined by observation at 3-h intervals from 09:00 h on Day 21 until the birth. A dim red flash-light was used during the hours of darkness. The time of observation just after the delivery of the first young was designated as the time of birth, irrespective of litter size. However, females with <4 young were excluded, because a delay of parturition has been reported for rats producing small litters (Sherwood *et al.*, 1985).

Experiment 1. Three different lighting conditions were used for pregnant Sprague-Dawley rats. In Group 1 (82 rats) the laboratory photoperiod (14L:10D; lights on at 05:00 h) was retained throughout pregnancy. For Group 2 (50 rats), the lighting regimen was kept at 14L:10D but lights were on from 17:00 to 07:00 h from Day 7 of pregnancy. The 48 rats in Group 3 were kept in constant darkness from Day 7 of pregnancy. A fourth group of 66 Wistar rats were maintained in 14L:10D (lights on at 05:00 h) throughout pregnancy. In each group, 6–8 rats were supplied to take the serial blood samples, and the remaining rats were used only to determine the time of birth.

Experiment 2. Two groups of Sprague-Dawley rats were maintained under the reversed lighting condition described above from Day 7 of pregnancy. With Group 1, on Day 20 of pregnancy the lights were switched on 8, 5 or 2 h earlier or 5 or 8 h later. In each light regimen, blood was taken from 7 rats at 4-h intervals for 24 h starting at 11:00 h on Day 21. With Group 2, 52 rats were injected intraperitoneally (i.p.) with pentobarbitone sodium (Nembutal; Abbott Laboratories, North Chicago, IL 60064, U.S.A., 31.5 mg/kg body wt) at 12:00, 15:00, 19:00, 21:00 or 23:00 h on Day 21. As control, 20 rats were injected with saline (9 g NaCl/l) at the same times. At 15:00, 19:00 or 23:00 h on Day 21, 24 rats were anaesthetized with ether. Blood samples were taken at 4-h intervals from 11:00 h on Day 21 to 15:00 h on Day 22. Then, the time of parturition was observed.

Experiment 3. Bilateral lesions of the SCN were made on Day 13 or 14 of pregnancy by an anodic direct current at stereotactically determined co-ordinates obtained from a rat stereotaxic atlas (Pellegrino & Cushman, 1967). To avoid deposits of Fe^{2+} in the lesion area, the stainless-steel electrode with 1 mm platinum (0.4 mm diameter) at the tip was insulated with epoxylite except for 0.2–0.3 mm at the tip. Rats were anaesthetized with i.p. pentobarbitone sodium and placed horizontally in a stereotaxis apparatus. The midline of the earbars served as a reference for the anterior-posterior positioning. The stereotaxic co-ordinates for the SCN electrode were set at 6.8 mm rostral, ± 0.35 mm lateral from the midline and 9.1 mm below from the top surface of the skull. In preliminary tests, when a direct current of 2 mA for 20 sec was used, a large lesion including the SCN was observed, and many rats did not litter (dead young) until Day 25. In this study, therefore, to make a small lesion including the SCN, a direct current of 1.0 mA for 20 sec was used. For sham-operations, the electrode was inserted into 7 rats but the current was not applied. To examine the elimination of circadian rhythm by the SCN lesion, patterns of drinking activity were determined at 4-h intervals for 24 h starting at 11:00 h on Day 19 by the method described previously (Sasaki *et al.*, 1984). The operated rats were used to determine the time of parturition. Lesions were verified at the end of the experiment by microscopic examination of serial sections.

Progesterone assay and statistical analysis. Progesterone was extracted from whole blood with petroleum ether, to remove corticosterone (mean recovery rate of progesterone extraction was 94%). The extracts were subjected to radioimmunoassay (Murakami *et al.*, 1979, 1982) using a specific antibody for progesterone. The steroids which showed any significant reactivity other than progesterone were corticosterone (1.3%) and 17 α -hydroxyprogesterone (7.6%). The minimum detectable level was 15 pg/tube. The intra-assay and inter-assay variances were 4.2% ($n = 5$) and 7.5% ($n = 6$), respectively. The difference in the patterns of progesterone concentrations between two groups was analysed by analysis of variance. The distributions of time of birth were compared by χ^2 analysis, or Student's t test after angular transformation.

Results

Experiment 1

The distribution of time of birth of rats in different photoperiods is presented in Fig. 1. All distributions differed significantly from each other ($P < 0.01$). In Group 1, most births occurred at 09:00–15:00 h during the light period on Day 23 in Sprague–Dawley rats (Fig. 1a). Some gave birth

Fig. 1. Incidence of time of parturition in Sprague–Dawley (a,b,c) and Wistar (d) rats (Exp. 1) kept in a lighting regimen of 14L:10D; lights on 05:00–19:00 h (a,d) or 17:00–07:00 h (b). In (c), rats were kept in continuous darkness. Half circle and complete circle symbols represent the time of parturition in 1 and 2 rats, respectively. Time of birth was determined at 3-h intervals.

during the light phase on Day 22, but most did not produce litters during the dark period. In Group 2 rats, parturitions again occurred in the light period between 23:00 h on Day 22 and 05:00 h on Day 23 (Fig. 1b). In Group 3, births occurred at all times on Day 22, 23 and 24 (Fig. 1c). Many rats gave birth on Day 23. There were two main parturition times in Group 4 rats (Fig. 1d), one being the light period on Day 22, and the other the light period on Day 23; only 7 out of 52 rats gave birth during the dark period. Mean ($\pm$ s.e.m.) litter size was 13.6 ± 2.3 and 9.8 ± 1.6 in Wistar and Sprague–Dawley rats, respectively.

Peripheral blood progesterone concentrations in individual rats during the pre-partum period are shown in Fig. 2. Individual progesterone concentrations declined rapidly from about 40 ng/ml

Fig. 2. The pre-partum decrease of blood progesterone concentrations in individual Sprague-Dawley (a,b,c) and Wistar (d) rats in Exp. 1. Several blood samples were taken individually at 4-h intervals. Open circles represent the time of birth in rats from which samples were taken.

at 07:00 or 11:00 h to basal levels at 11:00 or 15:00 h during the light period on Day 22 in Group 1 (Fig. 2a). In Group 2, the decrease occurred between 19:00 and 23:00 h during the light period on Day 21 (Fig. 2b). No influence of the serial sampling on the time of birth was observed in Groups 1 and 2. However, progesterone decrease occurred independently of time of day in Group 3 rats (Fig. 2c). The rate of decline progesterone values in Group 3 rats was more gradual than in Group 1 and 2. In Group 4, the progesterone decrease was observed independently of time of day between 11:00 h on Day 21 and 11:00 h on Day 22 (Fig. 2d).

Experiment 2

An advancement of the time of lights-on on Day 21 did not influence the time of progesterone decrease or the time of birth (Fig. 3a). However, when the time of lights-on was advanced by 8 h, a significant difference was observed in the time of birth and progesterone profile of ($P < 0.05$), relative to those in other groups (Fig. 3a). When the time of lights-on was delayed for 5 or 8 h, no significant differences were observed, relative to controls; in both the progesterone decrease occurred during the dark period (Fig. 3b).

Fig. 3. Effect of change of photoperiod on Day 21 on pre-partum decrease of blood progesterone concentrations in Sprague-Dawley rats kept under reversed lighting conditions (Exp. 2). Time of lights-on was advanced for 8 (○), 5 (□) or 2 h (△), or was delayed for 5 (■) or 8 h (▲) on Day 21. The results are presented as mean $\pm$ s.e.m. of 7–8 rats. Time of birth is indicated by a symbol matching those for the progesterone values.

Fig. 4. Effect of treatment with pentobarbitone sodium on the pre-partum decrease of blood progesterone concentrations in Sprague-Dawley rats kept under a reversed lighting regimen (Exp. 2). Pentobarbitone sodium was injected i.p. at the times indicated in figure. Values are presented as mean $\pm$ s.e.m. for 10–11 rats.

Table 1. Effect of injection of pentobarbitone sodium on the time of birth, determined at 3-h intervals, in Sprague–Dawley rats

Treatment	Time of treatment (h)	No. of rats	Day 22		Day 23				
			21:00–24:00 h	03:00 h	06:00 h	09:00 h	12:00 h	15:00 h	19:00 h
Saline*		20	7	12	1	—	—	—	—
Ether	15:00	6	2	3	1	—	—	—	—
	19:00	10	3	6	1	—	—	—	—
	23:00	8	4	4	—	—	—	—	—
Pento-barbitone sodium	12:00	10	2	8	—	—	—	—	—
	15:00	10	0	2	2	2	3	1	—
	19:00	11	0	1	2	4	4	—	—
	21:00	11	0	0	2	3	4	2	1
	23:00	10	2	3	2	3	—	—	—

*Injected to each of 4 rats at 12:00, 15:00, 19:00, 21:00 and 23:00 h, and pooled data are presented.

Fig. 5. Time of parturition in rats with a complete SCN lesion (●), a partial SCN lesion (▲) or an intact SCN (○).

In the rats treated with pentobarbitone sodium, complete recovery, judged by inspection, was observed 3–4.5 h after anaesthesia. The litter size was normal in rats anaesthetized with pentobarbitone (9.4 ± 2.2) or ether (9.6 ± 2.7). The treatment with pentobarbitone at 15:00, 19:00 or 21:00 h, but not 12:00 or 23:00 h, on Day 21 resulted in delay of the time of the progesterone decrease (Fig. 4) and in the time of birth, except for the group injected at 12:00 h (Table 1). The ether anaesthesia did not cause any significant effect on the time of parturition (Table 1).

Experiment 3

The 24-h pattern of water intake showed no rhythmicity in rats with complete SCN lesions, compared with that in control rats and those with partial SCN lesions. Histological examination indicated that 55% rats had complete SCN lesions, 35% partial lesions and 10% were unsuccessfully lesioned. Complete lesion of the SCN resulted in elimination of light-dependent parturition (Fig. 5b). Rats gave birth independently of time of day between Day 21 and 23. However, a strong tendency to advancement of the time of birth was observed, relative to that in control rats. Many rats with partial or unsuccessful lesions gave birth at the usual time (Fig. 5).

Discussion

Most births occurred between 09:00 and 15:00 h during the light period on Day 23 in Sprague–Dawley rats maintained under the usual light–dark regimen. When the photoperiod was reversed

on Day 7, parturition occurred between 23:00 and 05:00 h on Day 22–23. These results may indicate that parturition generally occurs during the light period. Lilit & Yeager (1974) demonstrated that time of blastocyst implantation was influenced by the photoperiod. In this study, however, the time of parturition may not be associated with the time of implantation, since the reversed photoperiod was given after the expected day of implantation (Day 5 of pregnancy). Lincoln & Porter (1976) and Sherwood *et al.* (1983) reported that advancement of photoperiod near mid-pregnancy advanced the time of birth. These results, including our observation, suggest that the time of parturition is closely associated with photoperiod.

Lincoln & Porter (1979) demonstrated that rat exposed to continuous darkness on only Day 23 gave birth during the same period as did controls. In this study, no extensive influence was observed on the time of birth even when the time of lights-on was changed on Day 21. Furthermore, the rats exposed to continuous darkness for a long time gave birth independently of time of day between Days 22 and 24. These results suggest that light-dependent parturition may be associated with an endogenous circadian system, and that light may be a strong entrainer of the time of parturition, but not the main trigger.

There is evidence that light is mediated by retinohypothalamic projection to the SCN (Moore & Eichler, 1972) which has been proposed to be an oscillator of endogenous circadian system (Moore, 1973; Inouye & Kawamura, 1979; Rusak & Groos, 1982). Complete lesion of this site scattered the time of parturition, suggesting that the SCN may be involved in determination of the time of birth. Lincoln & Porter (1979) reported that the lesion of the hypothalamus including the SCN caused two notable changes in the timing of birth, some 29% of animals failing to give birth and the remaining giving birth independently of time of day. Although the reason for failure of birth by lesion of the SCN is not clear, large lesions of the hypothalamus may be the cause (Honma *et al.*, 1984). Parturition in the rat is brought about by intricate mechanisms (Fuchs, 1973; Thorburn & Challis, 1979) involving adrenal function (Bosc & Nicolle, 1979, 1980), pituitary hormones (Fuchs *et al.*, 1974; Gordon & Sherwood, 1985), relaxin (Downing & Sherwood, 1985), prostaglandins (Dukes *et al.*, 1974; Labhsetwar & Watson, 1974; Strauss *et al.*, 1975; Buckle & Nathanielsz, 1975), ovarian steroids (Catala & Deis, 1973; Thorburn & Challis, 1979), or relationship between fetus and mother (Sherwood *et al.*, 1985). The mechanism affected by lesion of the SCN is uncertain. For example, lesion of the SCN eliminates the circadian rhythm of adrenocortical steroid (Moore & Eichler, 1972) which is considered to be one of the triggers of parturition (Thorburn & Challis, 1979).

The pre-partum decrease of blood progesterone in Sprague–Dawley rats occurred rapidly between 07:00 and 15:00 h during the light period in the usual lighting condition. When the photoperiod was reversed on Day 7, the progesterone decrease also occurred in the light period, between 19:00 and 23:00 h on Day 21. These results are consistent with a view that the progesterone decrease is correlated with daily photoperiod, as Sherwood *et al.* (1983) have demonstrated. However, it is not clear whether the light-dependent progesterone decrease is associated with an endogenous circadian system. This study provides two pieces of evidence: (1) the decrease occurred independently of time of day in rats exposed to continuous darkness for a long time (Fig. 2c), and (2) change of the lighting regimen on Day 21 only had very little effect on the progesterone decrease on Day 21 (Fig. 3). These results suggest that the progesterone decrease may also be closely associated with an endogenous circadian system. The reason why the time of progesterone decrease was delayed by advancement of lights-on for 8 h is not known. However, one possibility is that a phase shift may be present: time-dependent phase shifts by a light pulse have been reported for free-running rats (Daan & Pittendrigh, 1976).

In Wistar rats, the distribution of parturition was biphasic, as observed previously (Bosc & Nicolle, 1980), and suggests that parturition in Wistar rats is also associated with photoperiod. However, the progesterone decrease occurred independently of time of day. Although the reason for this difference between Wistar and Sprague–Dawley rats is not clear, the sensitivity to or dependence of the probable luteolytic factor with photoperiod may be different.

In Sprague–Dawley rats, the fact that the progesterone decrease occurred during a limited period of the day suggests that rapid luteolysis may also occur during a limited period. The progesterone decrease was delayed by treatment with pentobarbitone sodium at 15:00, 19:00 or 21:00 h, but not at 12:00 or 23:00 h, on Day 21, suggesting that the nervous system may be associated with the decrease between 15:00 and 23:00 h on Day 21. The nature of the putative luteolytic process associating with circadian rhythm (Thorburn & Challis, 1979) and processes involved with a possible direct effect on the ovary of pentobarbitone are unknown.

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