EFFECT OF OESTROGEN UPON
PARTURITION, MATERNAL BEHAVIOUR AND
LACTATION IN OVARIECTOMIZED
PREGNANT RATS

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SUMMARY

Ovariectomy of rats on day 20 of pregnancy impaired parturition and lactation. All rats showed prolonged delivery with many foetuses born dead and the mothers were unable to rear the young. Treatment with oestradiol benzoate (0.5 or 1 µg) in a single dose permitted normal delivery and lactation when the hormone was injected immediately after ovariectomy. Oestrogen administered the day after ovariectomy was not effective. The administration of prolactin and corticotrophin to ovariectomized pregnant rats did not prevent abnormal parturition but lactation improved in 50% of the mothers. A group of animals ovariectomized on day 20 was treated with oxytocin every 2 h on day 22 starting at 08.00 h. This treatment did not facilitate parturition and lactation. Maternal behaviour, which was always present in the other groups, was also impaired by this treatment. When ovariectomy was performed on the evening of day 21 of pregnancy, a partial impairment of parturition was observed but lactation was normal in seven out of eight rats. The results indicate that oestrogen is essential near term for normal parturition and lactation. The absence of the steroid may make the uteri less sensitive to oxytocic substances and also affect milk ejection.

INTRODUCTION

Parturition marks the culmination of organic changes occurring during pregnancy in which the endocrine system plays an important role. Recent studies have shown changes in the secretion of ovarian hormones and prolactin at the end of pregnancy (Grota & Eik-Nes, 1967; Yoshinaga, Hawkins & Stocker, 1969; Amenomori, Chen & Meites, 1970; Wiest, 1970). The endocrine mechanisms involved in the termination of pregnancy seem to be essential for the occurrence of parturition, lactogenesis and the expression of maternal behaviour and nursing. Several authors performed ovariectomy at the end of pregnancy in order to see if the rats underwent normal parturi-

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tion and showed maternal behaviour in the absence of ovarian hormones (Jost, 1959; Moltz & Wiener, 1966; Csapo, 1969; Terkel, 1970). In many animals ovariectomy led to difficult parturition and inability to rear the young. In the present work we tried to determine the hormones which are necessary for normal parturition, maternal behaviour and lactation in the absence of the ovaries.

MATERIALS AND METHODS

White primiparous rats (Instituto Strain), 3–4 months old, were used. They were placed with a male rat during the night following pro-oestrus. The next morning was counted as day 0 of pregnancy if spermatozoa were found in the vaginal smears. In our colony, rats usually deliver on day 22. Rats were kept in groups of five to six in special wire cages in a constant-temperature room (24 °C) in which the lighting schedule was 14 h light (06.00 to 20.00 h): 10 h dark (20.00 to 06.00 h). In all pregnant rats except one group, bilateral ovariectomy was performed through dorso-lateral incisions under ether anaesthesia, at noon on day 20 of pregnancy. In the remaining group ovariectomy was performed on day 21. In the control rats sham operations were performed in a similar way without removing the ovaries. After operation the rats were left in single cages specially designed for maternal behaviour studies. The cages were made of aluminium with a wire mesh cover and divided in two boxes (one smaller than the other) by a wire mesh partition. Both compartments were connected by a small hole made in the wire mesh (6 × 10 cm). The majority of the mothers built the nest in the small box.

The experimental animals were divided into ten groups and treated as follows: Group 1, Sham operation on day 20 of pregnancy; Group 2, Ovariectomy on day 20; Group 3, Ovariectomy on day 20 and treated with oxytocin (100 µu/rat every 2 h, i.p.) on day 22 of pregnancy starting at 08.00 h; Group 4, Ovariectomy on day 20 plus a single dose of 0-25 µg oestradiol benzoate (EB) s.c. immediately after operation; Group 5, Ovariectomy on day 20 plus a single dose of 0-5 µg EB s.c. after operation; Group 6, Ovariectomy on day 20 plus EB in a single dose of 1 µg after operation; Group 7, Ovariectomy on day 20 plus EB in a dose of 0-5 µg on day 21 at 20.00 h; Group 8, Ovariectomy on day 20 and treated with 1 mg prolactin and 2-5 i.u. corticotrophin (ACTH) s.c. 12 h after operation; Group 9, Similar to group 8 but prolactin and ACTH administered 34 h after ovariectomy; Group 10, Ovariectomy on day 21 of pregnancy at 20.00 h.

Parturition, maternal behaviour and nursing were the principal factors considered in the present study. In all cases parturition was checked every 2 h from 08.00 h to midnight. When it took place on day 22 of pregnancy, its duration was not more than 2 h and no more than three pups were born dead or were eaten by the mother. In the group classified as having abnormal parturition the events of delivery did not follow the pattern described by Rosenblatt & Lehrman (1963); delivery was prolonged (most of the rats took more than 24 h to deliver all the foetuses), more than three pups were born dead or eaten by the mother and she also failed to eat the placentae and clean the pups.

The following parameters of maternal behaviour were studied: nest building, retrieving of the pups, licking the litter, and the nursing position, according to the descriptions of Rosenblatt & Lehrman (1963). Observations were made every 2 h.
Oestrogen and parturition in rats

from 08.00 h to midnight. When the number of live pups was less than five, foster litters were given to the mother immediately after completion of delivery.

The nursing position, presence of milk in the stomach of the young, growth of the litter and the appearance of the nipples of the mother were observed for the evaluation of normal lactation. Both mothers and litters were checked twice a day.

The hormones used were: synthetic oxytocin (Syntocinon, Sandoz Argentina); prolactin (NIH-P-S-9, ovine); corticotrophin (ELEA, Argentina); and oestradiol benzoate (Progynon, Schering Argentina); 0·9% NaCl solution was the vehicle used for oxytocin and prolactin, and peanut oil for oestrogen.

RESULTS

Effect of ovariectomy performed on day 20 of pregnancy

Control sham-operated rats showed normal parturition, maternal behaviour and lactation. The nine ovariectomized rats showed abnormal parturition with prolonged delivery, seven displayed maternal behaviour, one died during parturition and the other did not show maternal behaviour. Lactation was absent in all rats, but in the majority of the mothers the nipples bore evidence of intense suction.

Treatment with oxytocin

In order to see if the disturbance of normal parturition after ovariectomy was due to a lack of oxytocin release, 11 rats ovariectomized on day 20 were injected with 100 mu. oxytocin every 2 h starting at 08.00 h on day 22. None of the rats delivered normally and one rat died during parturition. Only two rats showed maternal behaviour and only one was able to feed the pups.

Treatment with oestrogen

Four groups of ovariectomized pregnant rats were treated with different doses of oestradiol benzoate administered immediately after operation. When a dose of 0·25 µg EB was used the results were similar to that observed in the ovariectomized rats without treatment. None of the rats delivered in a normal way. Maternal behaviour was present in five out of six rats and normal nursing was evident in only one rat. Different results were obtained by increasing the dose of EB to 0·5 µg. In this group six out of ten rats showed normal parturition, three had abnormal delivery and one died during parturition. The nine rats showed maternal behaviour and were able to nurture the young. When the dose of EB was increased to 1 µg, in a group of eight rats, six showed normal parturition but the other two showed some disturbances. Maternal behaviour and nursing were completely normal in all rats and the litters grew normally.

In order to see if the effect of oestrogen depends on the time of administration, a group of six rats ovariectomized on day 20 was injected with 0·5 µg EB at 20.00 h on day 21. In this group all rats displayed abnormal parturition. In spite of all the rats having normal maternal behaviour, only two mothers were able to rear the pups to weaning.
Treatment with prolactin and corticotrophin

Considering the well-known action of oestrogen in inducing prolactin release (Desclin, 1956; Ramirez & McCann, 1964; Deis, 1967a, b; Zambrano & Deis, 1970) and also the fact that immediately before parturition prolactin secretion occurs (Grosvenor & Turner, 1960; Amenomori et al. 1970) a group of pregnant rats was treated with 1 mg prolactin and 2·5 i.u. ACTH 12 h after ovariectomy. ACTH was used together with prolactin since it was observed that in hypophysectomized-ovariectomized pregnant rats, prolactin induced lactogenesis only when administered in combination with corticotrophin (ACTH) (N. Alonso & R. P. Deis, unpublished). Table 1 shows that none of the six treated rats had normal delivery but all showed normal maternal behaviour. The litters of three mothers obtained milk and grew normally. The other three mothers presented sucked nipples but the pups did not obtain milk. In another group of six rats in which both hormones were injected 34 h after ovariectomy (i.e. day 21 of pregnancy) a similar result was obtained: all rats had undergone abnormal parturition, showed good maternal behaviour and only 50% of the mothers were able to nurse their litters.

Table 1. Effect of oxytocin, oestrogen, prolactin and corticotrophin (ACTH) on parturition, maternal behaviour and lactation in ovariectomized pregnant rats

<table>
<thead>
<tr>
<th>Group*</th>
<th>No. of rats</th>
<th>Parturition</th>
<th>Maternal behaviour</th>
<th>Lactation‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normal</td>
<td>Abnormal</td>
<td>Present</td>
</tr>
<tr>
<td>1. Control (sham-operated)</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>2. Ov. on day 20</td>
<td>9</td>
<td>0</td>
<td>8+1†</td>
<td>7</td>
</tr>
<tr>
<td>3. Ov. + oxytocin</td>
<td>11</td>
<td>0</td>
<td>10+1†</td>
<td>2</td>
</tr>
<tr>
<td>4. Ov. + 0·25 µg oestrogen</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>5. Ov. + 0·5 µg oestrogen</td>
<td>10</td>
<td>6</td>
<td>3+1†</td>
<td>9</td>
</tr>
<tr>
<td>6. Ov. + 1·0 µg oestrogen</td>
<td>8</td>
<td>6</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>7. Ov. + 0·5 µg oestrogen</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>on day 21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Ov. + 1·0 mg prolactin + 2·5 i.u. ACTH on day 20</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>9. Ov. + 1·0 mg prolactin + 2·5 i.u. ACTH on day 21</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>10. Ov. on day 21</td>
<td>9</td>
<td>5</td>
<td>3+1†</td>
<td>7</td>
</tr>
</tbody>
</table>

* Ov. = ovariectomy. See text p. 220 for full description of treatment groups.
† One rat experienced serious difficulties in parturition and was killed on day 24.
‡ Normal = normal nursing; absent = failure to nurse.

Effect of ovariectomy on day 21 of pregnancy

To determine if ovariectomy should be performed at a precise moment of pregnancy in order to obtain the above-mentioned disturbances, nine pregnant rats were ovariectomized on day 21 at 20.00 h. Of these nine rats, five delivered normally, in four delivery was prolonged and in one of these death occurred during parturition. Seven out of the eight rats showed good maternal behaviour and were able to rear the litter to weaning.
DISCUSSION

The present findings show clearly that ovariectomy, when performed at noon on day 20 of pregnancy, produces a series of impairments in parturition and lactation. Many foetuses were born dead showing signs of compression. Control, sham-operated rats on the contrary behaved as normal pregnant rats. Jost (1959) described abnormal parturition in pregnant rats ovariectomized on day 19 or 20 of pregnancy. In his work only ten rats out of 23 were able to rear the young. Moltz & Wiener (1966) performed ovariectomy plus Caesarean section on day 21 and observed that only 50% of the animals showed maternal behaviour with foster litters and were able to rear the young to weaning. On the other hand Moltz, Robbins & Parks (1966) found that Caesarean section does not affect maternal behaviour regardless of difficulty in parturition in pregnant rats ovariectomized on the evening of the 20th day, but only seven out of 20 rats were able to nurse the young. Csapo (1969) reported delayed and arrested labour in ovariectomized rats, and in experiments in vitro a failure in excitability and pharmacological reactivity of the uteri of these ovariectomized animals. Oestrogen substitution therapy restored normal excitement and reactivity, and also prevented arrested labour. All these findings favour an essential role of the ovarian hormones at the end of pregnancy for normal parturition and consequently normal lactation.

In pregnant rats, Yoshinaga et al. (1969) described a rapid increase in oestrogen concentration in ovarian venous plasma near term. The highest value was obtained on the day of parturition and fell to non-detectable levels on the day after delivery. The physiological effect of the rise of oestrogen at the end of pregnancy could be referred in part to an increase in the sensitivity of the rat uterus to oxytocic substances (i.e. oxytocin, prostaglandins) and also to induce prolactin release which is necessary for lactogenesis. The effect of oestrogen on prolactin release is well established (see Zambrano & Deis, 1970, for references). In the present work release of prolactin is not prevented since ovariectomy in pregnant rats induces lactogenesis 24 h later (Deis, 1968; N. Alonso & R. P. Deis, unpublished results) and plasma prolactin levels increase significantly 6–8 h after operation (R. P. Deis & N. T. Vermouth, unpublished results). Lack of oestrogen in the ovariectomized pregnant rats induces uterine hypomotility which may impede respiratory exchange and lead to foetal anoxia. The inability to deliver, or delay in delivery of, the foetuses after the placenta has been dislodged may lead to foetal asphyxia. On the other hand abnormal parturition will produce intense stress which may affect oxytocin release and milk ejection. Stress has been shown to prevent oxytocin release in lactating rats (Cross, 1955; Taleisnik & Deis, 1964). It is interesting to note that several ovariectomized rats had nipples which showed clear evidence of intense sucking, indicating that failure of lactation was not due to a lack of suckling stimulus.

The fact that administration of oestrogen (0.5 or 1 µg) after ovariectomy induced normal parturition and lactation indicates the essential role of oestrogen near term. The results obtained in the group of rats ovariectomized on day 20 of pregnancy and treated with oestrogen the following day indicate that the hormone is effective when injected on day 20 of pregnancy, but not later. Also considering that when ovariectomy was performed on day 21 of pregnancy, five out of nine rats showed normal
parturition and seven out of eight mothers were able to nurse, we can postulate that oestrogen secreted on day 20 seems to be sufficient to induce normal parturition and lactation. Jost (1959) and Terkel (1970) showed that the percentage of normal parturition increased when the pregnant rats were ovariectomized near term.

In the two groups of ovariectomized rats treated with prolactin and ACTH, 50% showed normal lactation but none of the rats showed normal parturition. Nevertheless, maternal behaviour was evident in all rats. It seems that both hormones in some way favoured lactation.

The group of ovariectomized rats treated with oxytocin showed abnormal delivery and virtual absence of maternal behaviour. This result indicates that the uterus did not respond to oxytocin, and the administration of the hormone probably increased the stress in parturition which may have affected maternal behaviour. Gonadectomy in normal female or male rats induces a high oxytocin concentration in the posterior lobe but it is not known if this increment is due to a reduction in the release of the hormone. Oestrogen was capable of restoring oxytocin to normal values in these rats (Deis, 1959). On the other hand Wiesner & Sheard (1933) showed that ovariectomy immediately after delivery did not affect maternal behaviour and lactation. In the group of rats ovariectomized on day 21 of pregnancy in the present work seven out of eight rats reared their young. These findings demonstrate clearly that ovariectomy did not affect lactation through an effect on oxytocin release.

We can conclude that pregnant rats need oestrogen at the end of pregnancy. The absence of the hormone reduced the sensitivity of the uterus and thus impaired expulsion of the foetuses. This increases parturitional stress and may prevent oxytocin release, necessary not only for parturition but also for milk ejection. That factors other than stress may affect oxytocin release, however, cannot be excluded.

REFERENCES


Oestrogen and parturition in rats


