Influence of the environmental temperature 
on the post-partum testosterone surge in the rat

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Abstract. In the neonatal male rat, a rapid and transient increase in serum testosterone occurs about 2 h after birth. This post-partum testosterone surge (PPTS) has been implicated in the masculinization and defeminization of the central nervous system. The present study shows that environmental temperature can have a profound influence on the PPTS. Male rats were delivered from their mothers by caesarean section on day 22 of gestation. Immediately thereafter, neonatal males were placed at an ambient temperature of either 18, 21, 24 or 30°C. With 2 h of exposure, the body temperature was in close correspondence with the ambient temperature. The PPTS was clearly abolished in the pups exposed for 2 h at either 18 or 21°C. The effect of temperature was reversible: by placing pups at either 18 or 21°C for 2 h after delivery, and then rewarming by placing them with a foster mother, the PPTS was delayed until 4 h after birth, i.e. 2 h after the beginning of rewarming. Thus, environmental cooling appears to retard the development of neural and/or endocrine systems mediating the PPTS. Aberrant maternal care which would produce substantial cooling of the male pups would be expected to affect the PPTS, which in turn might affect the sexuality of male progeny.

Testicular secretions during the perinatal period are critical for the sexual differentiation of the male rat (Gorski 1971). In the newborn male, a sudden peak in serum LH is followed by a rapid rise in serum testosterone at about 2 h after birth (Roffi et al. 1977; Corbier et al. 1978; Pang et al. 1979; Slob et al. 1980). This testosterone surge appears to be involved in the differentiation of the central nervous system, influencing both defeminizing (Corbier et al. 1983; Corbier 1985) and masculinizing processes (Roffi et al. 1986).

The activity of the pituitary-testicular axis of the newborn rat is theoretically susceptible to modification by both internal and external variables. We have already shown that neither the fall in blood level of progesterone in the newborn (Rhoda et al. 1984) nor a change in ambient lighting influences the neonatal testosterone surge in the neonatal male rat (Roffi et al. 1985). The purpose of the present study was to investigate the effect of environmental cooling on the neonatal testosterone surge in newborn male rats.

Materials and Methods

Animals
The experiments were carried out on albino rats from the Sherman strain bred in our laboratory. Conditions of breeding and reproduction have been previously described (Corbier & Roffi 1978). The animals were housed under controlled conditions of lighting (lights on 06.00–20.00 h). The temperature of the animal room was kept at 22 ± 1°C. The onset of gestation was defined as the estimated hour of ovulation (02.00 on day 0), during the night of cohabitation. The pregnant females were recognized on day 14 by palpation and caged individually. In our colony, parturition usually occurs either on day 21 or 22 of gestation.
Experimental procedure
Experiment 1: The mother was killed by cervical dislocation on day 22 of gestation, between 09.00 and 11.00 h and, after laparotomy, the foetuses were immediately removed from the uterine horns (0 h). The female pups were discarded and the male pups were dried on filter paper and were left undisturbed for a period of 2 h, in an open polystyrene box (12 × 123 cm), at one of the following ambient temperatures: 30, 24, 21 or 18°C. After 2 h, skin temperature of some of the males in each litter was measured with a skin probe on the left side of the body, and body temperature was measured with a subcutaneous probe. The males were then killed by decapitation and trunk blood was collected for serum testosterone assay.

Experiment 2: This experiment was performed in order to know whether cooling affects the serum testosterone levels in one-day-old rats. Spontaneous delivered males were left with their mother during 24 h and divided into two groups. One group was immediately decapitated for trunk blood collection; the other group was killed at 19°C for 3.5 h before being decapitated for testosterone assay.

Experiment 3: Caesarean-delivered male pups were placed at 18°C for a 2-h period. At the end of that period, some males were killed and trunk blood collected. Other males were given for nursing to a foster mother whose own pups had been withdrawn. The temperature under the mother was approximately 35°C. The pups were then killed for testosterone assay, at either 4, 6 or 8 h of age.

Testosterone assays
The blood from 3–5 pups was pooled to make a sample for testosterone assay. Samples were kept at 4°C for 24 h and serum was collected by centrifugation. Serum samples were stored at about −25°C until assays. Serum testosterone was determined by radioimmunoassay, according to a previously described method (Corbier et al. 1981). Briefly, an internal standard of approximately 2500 dpm tritiated testosterone ([1,2,6,7-3H]testosterone, SA 109 Ci/mmol, purchased from Amersham Corp, Arlington Heights, IL) was added to each 0.1 ml serum sample and was allowed to equilibrate at 37°C for 30 min. The samples were then adjusted to 1 ml with distilled water and extracted twice with 6 ml of diethyl ether. The organic phase was separated from the aqueous phase by centrifugation at 4000 rpm and was dried under a stream of nitrogen. The antiserum for testosterone was a result of immunization with testosterone-3β-carboxy-methoxime-BSA, and was provided by Dr Mouren (Roussel-UCLAF). This antibody cross-reacts principally with 5α-androstan-17β-ol-3-one (14%) and 5-androsten-3β,17β-diol (3%). The assay sensitivity was 4.2 ±0.1 nmol/tube. The intra-assay coefficient of variation was 9.7%; the inter-assay coefficient of variation was 12.6%. The results of the assays are expressed in nmol per 1 serum.

Statistical analyses
Between group comparisons were made with Student’s t-test for independent groups (two-tailed).

Results
In the first experiment, we separated caesarean-delivered male pups from their mothers and exposed them to one of 4 different ambient tem-

<table>
<thead>
<tr>
<th>Environmental temperature (°C)</th>
<th>No. of animals</th>
<th>Skin temperature (°C)</th>
<th>Body temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>17</td>
<td>18.1 ± 0.2</td>
<td>17.8 ± 0.2</td>
</tr>
<tr>
<td>21</td>
<td>19</td>
<td>20.6 ± 0.1</td>
<td>20.7 ± 0.1</td>
</tr>
<tr>
<td>24</td>
<td>14</td>
<td>22.8 ± 0.2</td>
<td>22.9 ± 0.2</td>
</tr>
<tr>
<td>30</td>
<td>15</td>
<td>29.9 ± 0.1</td>
<td>29.8 ± 0.1</td>
</tr>
</tbody>
</table>

Table 1.
Skin and body temperatures in 2 h old neonatal male rats born by caesarean section at term (day 22 of gestation) and exposed at 0 h to different temperatures. The values are the means ± sem.
Temporal changes of serum testosterone in neonatal male rats delivered by caesarean section on day 22 of gestation: influence of the rewarming of the pups by a foster mother. Full lines (●—●) indicate the temporal changes in male exposed to 18°C for 2 h and then placed with a foster mother. For comparison, broken lines (●—●) recall the temporal changes of serum testosterone levels in pups left with their mother (from Corbier et al. 1981). (N) = numbers of plasma pools assayed.

Fig. 2.

Inasmuch as the predictable rise in serum testosterone occurs normally in males maintained apart from a mother at ambient temperatures of 24°C or above (Fig. 1), it is clear that it is cooling rather than the absence of a mother per se which prevents the testosterone surge in males exposed to 21°C or below. Put in another way, under normal circumstances, a mother provides warmth and nutrition to newborn males; warming alone, even in the absence of the mother will support the development of the testosterone surge in the newborn male.

Discussion

Neonatal rats are essentially poikilothermic and are unable to regulate their body temperature (Gullick 1937; Brody 1943; Hill 1947). In caesarean-delivered males, environmental cooling quickly causes skin temperature to fall, and skin and body temperatures closely approximate the ambient temperature (Table 1). These results for newborns are in complete accord with results for one-day-old rat pups (Poczopko 1961).

Caesarean-delivered male rats placed with a foster mother show a dramatic rise in serum testosterone at about 2 h (Corbier et al. 1978). This rise is completely prevented in caesarean-delivered males maintained apart from a mother at an ambient temperature of 18 or 21°C (Fig. 1), or by placing the pups at 2°C on bed of ice (Hary et al. 1986).

Environmental cooling lowers body temperature but has no effect on the serum testosterone levels of one-day-old males. When environmental cooling prevents the rise in serum testosterone which would ordinarily occur at about 2 h after birth, it may do so by delaying the development of mechanisms ultimately responsible for the surge rather than acting on an already established hypophyseal-testicular axis.

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The rise in serum testosterone which normally occurs in males at 2 h after birth is preceded, and presumably caused, by an increase in serum LH within the first hour of extra-uterine life. Lowered body temperature produced by environmental cooling may inhibit the synthesis or the secretion of LH and/or attenuate the testosterone-secreting effects of LH on the neonatal testes. In this regard, it may be noted that lowering temperature decreases the rate of cerebral protein synthesis in 2-day-old rats (Dunlop et al. 1974). In the newborn, lowering temperature decreases the binding of FSH to specific receptors in the testes (Kukobawa & Ishii 1980); whether or not a similar effect can be obtained in the neonatal rat remains to be determined.

Cooling newborn males for 2 h and then placing them with nursing mothers delays the rapid rise in serum testosterone by a period approximately equal to that of cooling (Fig. 2). Although we cannot eliminate the possibility that this surge would have occurred even if the males had been cooled for a longer period of time, the temporal association between replacement with a nursing mother and the development of the testosterone surge suggests that the two are causally related. Environmental cooling seems to arrest development as inferred by the absence of a testosterone surge at 2 h; the rewarming which occurs as a consequence of replacing the pup with a nursing mother appears to 'restart' development as inferred by a testosterone surge 2 h later.

Not all mothers warm their litters immediately after parturition. Intervals of one hour or more may separate the time of birth of a newborn and the onset of retrieving, licking, nesting behaviours which promote pup warming. If the environmental temperature is 21°C or lower, the failure to warm pups could prevent or attenuate the testosterone surge which normally occurs in male rat pups during the first 2 h after birth. We have already demonstrated that preventing this surge by surgical castration has both feminizing (Corbier et al. 1983; Corbier 1985) and demasculinizing (Roffi et al. 1986) effects on sexual physiology and behaviour.

Temperature can influence the differentiation of the gonads in amphibians (Witschi 1914; Piquet 1930) and in reptiles (Piquet 1972), but whether or not preventing or delaying the testosterone surge in the neonatal male has consequences for the sexual differentiation of the male remains to be determined. We speculate, however, that at least some of the variability between normal males with respect to their potential for male and female sexual behaviour as adults may be due to environmental variation affecting body temperature during the early postnatal period.

Acknowledgments

This research was supported by Grant No. 91 86 010 from the Foundation de Recherche en Hormonologie and by Grant from the Foundation pour la Recherche Médicale.

References


