Genetic and hormonal aspects of male facilitation of PMSG-induced ovulation in immature mice

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Summary. Immature female mice of the SWR/J and C3H/1bg strains pretreated with PMSG showed higher incidence of ovulation when exposed to males of the same strain than did those kept isolated after weaning. Females of the C57BL/6Ibg strain were not affected. The numbers of eggs ovulated were also greater in SWR and C3H females than in the C57 females. When SWR/J females were killed after exposure to males for 2, 4 or 6 h, there was a 6-fold increase in LH levels at 6 h compared to values in isolated controls. It is suggested that adult male mice of some strains provide a stimulus which promotes release of LH in the immature female.

Introduction
It has been reported previously that male mouse pheromone(s) can influence the reproductive physiology of a female mouse. Male mouse primer pheromones have been shown to synchronize oestrus (Whitten, 1956), block implantation of the zygote (Bruce, 1959), increase the number of eggs released (Zarrow, Christenson & Eleftheriou, 1971), and accelerate sexual maturation (Vandenbergh, 1969) in the female mouse. All of these primer pheromonal effects have been assumed to occur via olfactory–hypothalamic gonadotrophin pathways (Whitten & Champlin, 1973). There have been only a few studies which have directly investigated the effect of male pheromones on gonadotrophin release (see Bronson & Stetson, 1973; Bronson & Desjardins, 1974; Bronson & Maruniak, 1976). Bronson & Desjardins (1974) and Bronson & Maruniak (1976) found that exposure to males as well as to their urine resulted in immediate elevation of LH values, and the present study was designed to investigate the relationship between this finding and the observation of “male pheromonal facilitation of ovulation in PMSG-primed immature female mice” (Zarrow et al., 1971).

The first experiment replicated the male facilitation of ovulation in PMSG-primed immature female mice and extended this to different strains. The second experiment tested the hypothesis that this effect is mediated by an increase in luteinizing hormone (LH) levels.

Materials and Methods

Experiment 1

Animals. Three inbred strains of mice were used: SWR/J breeding pairs were obtained from The Jackson Laboratories, Bar Harbor, Maine, and mice of the C3H/2Ibg and C57BL/6Ibg inbred strains were obtained from this Institute. Adult males, to which immature females were exposed, were at least 60 days old and of proven fertility (i.e. had sired litters). Immature female mice were weaned at 21 days of age and caged singly until tested.
All the animals were maintained and tested within the confines of the SPF laboratory. A 12 h light–dark cycle was maintained, with lights on from 06:00 to 18:00 h; temperature was 23.3 ± 1°C; and relative humidity was 45–55%. Animals were housed in standard stainless-steel cages. Food (Wayne Sterilizable Lab-Blox) and tap water were provided ad libitum.

 Procedures. Since preliminary results indicated that weight was an important factor in PMSG-induced ovulation, only females that weighed at least 7.0 g at the time of weaning were used. Littersmates were paired and matched by weight (±0.3 g) to control for litter and weight effects, and each member was then assigned to the exposure treatment or control group. At 09:00 h on the 24th day of age, each immature female was subcutaneously injected with 4 i.u. PMSG in 0-2 ml saline (9 g NaCl/l). On Day 26 of age, at 12:00 h (i.e. 51 h after the PMSG injection), females in the experimental group were exposed to males of the same strain for the rest of the experiment; the controls remained isolated. The experimental treatment involved transfer of a female to a cage with a 2.5 cm double wire partition in the centre. The female was housed on the side where an adult male mouse of proven fertility had resided for 2 days, while the male mouse was transferred to the opposite side of the partition. The width of the double wire partition was such that it prevented tactile stimulation between the pair. The fineness of the wire mesh (each square was 3 mm²) also reduced visual stimulation. Control mice were transferred to similar cages but no males were used. The experimental and control groups of each strain were housed in separate rooms with separate ventilation systems to eliminate confounding odours.

 At 09:00 on Day 27 (i.e. 72 h after the initial PMSG injection and 21 h after the start of male exposure), all females were weighed and then killed by cervical dislocation. Oviducts were removed and flushed, and the contents were examined under a dissecting microscope at ×40 magnification for the presence of ova in cumulus cells and for number of ova.

 **Experiment 2**

 Animals. Males and females of the SWR strain were used and were housed exactly as described for Exp. 1.

 Procedures. The procedures were the same as those of Exp. 1, except for the times for killing and collection of blood. Since it was not possible to obtain sufficient quantities of blood for radioimmunoassay by sequential sampling, groups of mice were killed by decapitation at 2, 4 and 6 h after the start of exposure to males (i.e. 53, 55 and 57 h after the PMSG injection). Groups of control mice were killed at the same times. Blood samples were allowed to clot overnight before centrifugation. Serum from each individual was then stored at −20°C until assay.

 The radioimmunoassay kit, kindly provided by Dr A. F. Parlow of the NIAMDD Hormone Distribution Program, has been previously examined and validated for assays of mouse LH (Beamer, Murr & Geschwind, 1972). The LH does not cross-react with PMSG in the radioimmunoassay (Dr A. F. Parlow, personal communication). The assay was performed by Dr Gordon Niswender and his staff at Colorado State University, Fort Collins, Colorado. The standard used was NIAMDD-rat-LH-RP1. Sensitivity of the assay was 218.29 pg/ml, and the limit of detection was 44.92 pg/ml.

 The data were analysed by χ², analysis of variance, or the Tukey B tests (Winer, 1971).

 **Results**

 **Experiment 1**

 Analysis of variance revealed significant strain differences in weight at 27 days of age (F = 188.55, d.f. = 2/112, P < 0.001). The mean ± s.e.m. weights of the SWR, C3H and C57BL mice when killed were 14.29 ± 0.09, 15.42 ± 0.15 and 11.44 ± 0.18 g, respectively. There was
no significant difference in body weight between ovulators and non-ovulators. As shown in Table 1, the proportion of females ovulating in response to exposure to males was greater if they were of the SWR or C3H strain. There were no strain differences in the occurrence of ovulation in the control groups.

Table 1. Effects of exposure of female mice to males of the same strain on occurrence of ovulation

<table>
<thead>
<tr>
<th></th>
<th>SWR/J</th>
<th>C3H/2Ibg</th>
<th>C57BL/6Ibg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested</td>
<td>No. ovulating (%)</td>
<td>No. tested</td>
</tr>
<tr>
<td>Exposed</td>
<td>26</td>
<td>22 (85)</td>
<td>18</td>
</tr>
<tr>
<td>Control</td>
<td>26</td>
<td>11 (43)</td>
<td>18</td>
</tr>
</tbody>
</table>

* $P < 0.05$; ** $P < 0.01$.

Data on the numbers of eggs ovulated were subjected to a two-way analysis of variance (exposure × strain) which allowed for unequal cell frequencies (Kim & Kohout, 1975). Females in the experimental groups released almost three times as many ova (10·06 ± 1·05) as did the control females (3·39 ± 0·54) ($F = 13·82$, d.f. = 1/71, $P < 0·001$). Strains also differed significantly in ova count. Significantly more ova were released by mice of the SWR (8·42 ± 1·22) and C3H (7·69 ± 1·15) strains than by those of the C57BL strain (3·30 ± 0·61) ($F = 8·20$, d.f. = 2/71, $P < 0·001$; Tukey’s B test). There was no strain × exposure interaction.

Experiment 2

Data on LH levels (see Table 2) were subjected to a two-way (exposure × duration) analysis of variance. Experimental SWR females had significantly higher levels of serum LH than did control females (96·30 ± 29·71 and 15·44 ± 2·64 ng/ml, respectively; $F = 9·06$, d.f. = 1/42, $P < 0·01$), although the LH levels were very variable in the exposed females. The time at which the females were killed (i.e. duration of exposure) had an effect on LH concentrations. Values at 14:00 h (18·25 ± 2·22 ng/ml) were significantly lower ($F = 4·15$, d.f. = 2/42, $P < 0·05$ (Tukey B test)) than at 18:00 h (109·10 ± 33·99 ng/ml). There was also an exposure × time interaction in that LH levels after exposure to male for 6 h were increased 6-fold compared to those in control females ($F = 3·11$, d.f. = 2/42, $P < 0·05$).

Table 2. Effects on LH concentrations (ng/ml) observed when SWR/J female mice were killed at different times of day after exposure to a male of the same strain at 12:00 h

<table>
<thead>
<tr>
<th>Time killed</th>
<th>Exposed</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:00 h</td>
<td>21·03 ± 3·27</td>
<td>15·46 ± 2·88</td>
</tr>
<tr>
<td>16:00 h</td>
<td>73·49 ± 59·99</td>
<td>7·03 ± 1·65</td>
</tr>
<tr>
<td>18:00 h</td>
<td>194·38 ± 53·24</td>
<td>23·82 ± 6·14</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m. for 8 mice at each time for each group.

Discussion

The results of both experiments confirm previous studies (Zarrow, Estes, Denenberg & Clark, 1970; Eleftheriou, Bailey & Zarrow, 1972; Eleftheriou, Christenson & Zarrow, 1973) that presence of an adult male of proven fertility can facilitate ovulation in an immature, PMSG-
been increase was contamination et Bartke, McClearn. Although exposure stimulation may ovulation, males C57BL/6Ibg Bruce, immature differences of primed females since body weight has been shown to be an important predictor of pubertal responsiveness of immature females to male exposure (Bronson & Desjardins, 1974). The normal relationship between body weight and puberty has not been established for the strains used in the present study. It is possible that the C57BL/6Ibg females may not have been as responsive to male exposure because they weighed less than did females of the other strains at this age of testing. Although strain differences in response to different doses of PMSG have been reported (Zarrow et al., 1971), it is unlikely that response to PMSG contributed to strain differences in the present study, since control females of the three strains responded similarly with respect to ovulatory behaviour. The occurrence of ovulation in the control females was probably related to LH contamination of the PMSG (Daniel, 1971) because a second gonadotrophin injection is usually used to induce ovulation after priming with PMSG.

It is more likely that differences in the pheromonal properties of the male account for the observed strain differences. The lack of male pheromonal activity in C57BL mouse strains has been found in other studies (Chipman & Bronson, 1968; Eleftheriou et al., 1972): e.g. there was facilitation of ovulation when C57BL/6J females were exposed to SWR/J males but no facilitation occurred when SWR/J females were exposed to C57BL/6J males (Eleftheriou et al., 1972). Some C57BL strains are testosterone-deficient (Bartke, 1974). Since several pheromonal effects have been found to be androgen-dependent (Whitten, 1966; Bronson & Whitten, 1968; Bruce, 1970; Eleftheriou et al., 1973; Vandenbergh, 1975), this could be the reason why C57BL/6Ibg males did not facilitate the occurrence of ovulation in females of the same strain or increase the number of ova released by females which did ovulate.

The results of Exp. 1 therefore support previous findings that male presence (presumably male pheromones) can facilitate PMSG-induced ovulation in immature females and that there are strain differences in facilitation. Mice of the SWR/J strain were used in Exp. 2 because the difference between the experimental and control females of that strain was greatest in Exp. 1, the LH levels after 6 h of male exposure being 6-fold greater than in control females. The variable LH levels of the exposed females were probably due to individual differences in responsiveness. When tactile stimulation was allowed, Bronson & Desjardins (1974) found a 4- to 5-fold increase in serum LH after 1–3 h of male exposure in young females experiencing a precocious puberty. Bronson & Maruniak (1976) found that exposure to male urine resulted in elevation of serum LH within 30 min.

Although the increased LH concentrations can account for the increase in occurrence of ovulation, it is uncertain whether the higher ovulation number is also a consequence. The male may also stimulate the release of FSH, which increases recruitment to the population of mature follicles. It is clear that male stimuli act directly on the secretion of LH, presumably by central stimulation of the hypothalamus, leading to release of LH-RH.

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References


Induced ovulation in immature mice


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