Parthenogenetic activation of mouse oocytes following avertin anaesthesia

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SUMMARY

Avertin anaesthesia induced mouse eggs to become activated parthenogenetically. An increasing incidence of activation was observed when females were anaesthetized 6.5, 9 and 13 h after ovulation, reaching a maximum of 45.8%. In a spontaneously ovulating group approximately 12.5% of all the eggs ovulated, or 27.3% of all the eggs activated evoked a decidual response, and the presence of implanting embryos was confirmed histologically. These findings demonstrate a new and simple method of inducing post-implantation parthenogenetic development in the mouse, and stress the necessity of taking into account the possible consequences of anaesthesia in the early post-ovulatory period.

INTRODUCTION

The induction and examination of mammalian parthenogenones could provide a valuable tool in the experimental analysis of fertilization and early development. This led me to pursue the chance observation that parthenogenetic development was initiated when mice were anaesthetized with avertin, a preparation of tribromoethanol dissolved in amylene hydrate, commonly employed as a general anaesthetic in animal practice.

It was already known that ether anaesthesia could initiate parthenogenetic development (Braden & Austin, 1954a), while heat shock (Braden & Austin, 1954b) and electric stimulation of the oviduct (Tarkowski, Witkowska & Nowicka, 1970; Witkowska, 1973a) could activate mouse eggs in vivo. Treatment of eggs in vitro with hyaluronidase (Graham, 1970; Kaufman, 1973), filtrates of sperm suspensions (Kaufman, 1973), and heat shock (Komar, 1973), are also capable of inducing parthenogenetic activation.

MATERIALS AND METHODS

Eight- to twelve-week-old (C57BI × A6G)F1 hybrid female mice were kept under controlled lighting conditions (dark period from 7 p.m. to 5 a.m.), and anaesthetized with an intraperitoneal injection of avertin at various times after spontaneous or induced ovulation. The standard dose of anaesthetic used was

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0.02 ml/g body weight of a freshly prepared 1:80 solution of avertin dissolved in 0.9 % saline.

Spontaneously ovulating females were mated to proven sterile vasectomized males, and anaesthetized at various times throughout the day on which the vaginal plug was observed (day 0 of pseudopregnancy). A second group of females was superovulated with 5 or 10 i.u. pregnant mares' serum gonadotrophin (PMSG), followed 48 h later by 5 or 10 i.u. of human chorionic gonadotrophin (HCG). A third group was mated with vasectomized males shortly after the HCG injection.

In the spontaneously ovulating females the time of ovulation was assumed to be approximately the middle of the dark period (Braden, 1957). The number of eggs ovulated spontaneously was 4.00 ± 0.27 (mean ± s.e.) per oviduct (Table 1). Ovulation occurred approximately 12 h after the HCG injection in the gonadotrophin-induced females.

Spontaneously ovulating females, previously mated to vasectomized males, were anaesthetized 4, 6.5, 9 or 13 h after midnight (the mid-dark point of the mouse photoperiod), while superovulated females were anaesthetized 20 or 25 h after HCG. Saline-injected controls and females mated but not anaesthetized were also examined.

Females were either killed 20-24 h after anaesthesia (group A) when their oviduct contents were examined (day 1), or on the morning of day 3 (group B) when their oviduct and uterine contents were examined, or on days 5, 6 or 7 (group C) to determine the number of decidua present.

**RESULTS**

In the spontaneously ovulating mice, mating to vasectomized males did not induce activation (Table 1, group 1), nor was activation observed in the females anaesthetized at 4 a.m. (group 2). An increasing incidence of activation was observed when females were anaesthetized 6.5 (group 3), 9 (groups 4 and 5) and 13 h after midnight (group 6), reaching a maximum of 45.8 % (group 6). Increasing the dose of anaesthetic (group 5) did not affect the activation frequency, but increased the incidence of degenerated eggs observed 20-24 h later. Nine out of 11 eggs in the 13-h group were at the 3- or 4-cell stage at the time of examination, suggesting that they underwent immediate cleavage. This is consistent with previous observations on the activation of eggs isolated approximately 13 h after ovulation (Kaufman, 1973).

A low rate of activation was observed at HCG + 25 h (group 8), though a high proportion of the eggs were found to be fragmenting. Reducing the dose of avertin by 50 % (group 9) failed to induce activation. Control females injected with saline at this time showed no evidence of activation (group 10).

Five spontaneously ovulating females of the 13-h group were killed in the morning of day 3 (group B). Two females had only non-activated eggs present.
Table 1. Reaction of mouse eggs 20–24 h after avertin anaesthesia

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time of anaesthesia/treatment</th>
<th>No. of oviducts</th>
<th>Fragmenting eggs</th>
<th>Degenerated eggs</th>
<th>Non-activated eggs</th>
<th>Activated eggs</th>
<th>Total eggs</th>
<th>% Activation</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Mated only (control)</td>
<td>6</td>
<td>—</td>
<td>—</td>
<td>29</td>
<td>—</td>
<td>—</td>
<td>29 0.0</td>
</tr>
<tr>
<td>2</td>
<td>Midnight* + 4 h</td>
<td>4</td>
<td>—</td>
<td>—</td>
<td>16</td>
<td>—</td>
<td>—</td>
<td>16 0.0</td>
</tr>
<tr>
<td>3</td>
<td>Midnight + 6.5 h</td>
<td>4</td>
<td>—</td>
<td>—</td>
<td>13</td>
<td>1</td>
<td>—</td>
<td>14 7.1</td>
</tr>
<tr>
<td>4</td>
<td>Midnight + 9 h</td>
<td>21</td>
<td>5</td>
<td>5</td>
<td>58</td>
<td>13</td>
<td>1</td>
<td>82 17.1</td>
</tr>
<tr>
<td>5</td>
<td>Midnight + 9 h (1.5 x standard dose)</td>
<td>8</td>
<td>—</td>
<td>19</td>
<td>3</td>
<td>5</td>
<td>—</td>
<td>27 18.5</td>
</tr>
<tr>
<td>6</td>
<td>Midnight + 13 h</td>
<td>6</td>
<td>2</td>
<td>—</td>
<td>11</td>
<td>2</td>
<td>2</td>
<td>7 45.8</td>
</tr>
<tr>
<td>7</td>
<td>HCG + 20 h</td>
<td>8</td>
<td>29</td>
<td>40</td>
<td>50</td>
<td>44</td>
<td>—</td>
<td>169 29.6</td>
</tr>
<tr>
<td>8</td>
<td>HCG + 25 h (standard dose of avertin)</td>
<td>8</td>
<td>70</td>
<td>8</td>
<td>53</td>
<td>6</td>
<td>2</td>
<td>6 145 9.7</td>
</tr>
<tr>
<td>9</td>
<td>HCG + 25 h (0.5 x standard dose)</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>52</td>
<td>—</td>
<td>—</td>
<td>56 0.0</td>
</tr>
<tr>
<td>10</td>
<td>HCG + 25 h (0.4 ml saline, control)</td>
<td>4</td>
<td>9</td>
<td>1</td>
<td>44</td>
<td>—</td>
<td>—</td>
<td>54 0.0</td>
</tr>
</tbody>
</table>

I. Spontaneous ovulation

II. Induced ovulation

* Midnight was the mid-dark point of the mouse photoperiod.
in their oviducts, while a total of 22 eggs were isolated from the remaining three females. Two eggs had degenerated, seven were non-activated, one was at the 2-cell stage, and twelve were at various cleavage stages between the 8-cell and 32-cell stages. The twelve cleavage embryos were examined by air-drying (Tarkowski, 1966), and haploid metaphase plates were observed in three embryos.

Eighteen females anaesthetized 13 h after midnight, and five at 20 h after HCG, were killed on days 5, 6 or 7. In the 13-h group, eight out of eighteen females had no implantation sites, while the remaining ten females had a total of 18 sites. Thus, in the females with implants, approximately 12.5% of all the eggs ovulated, or 27.3% of all the eggs activated, survived at least until the moment of implantation. This is similar to the implantation rate observed
Avertin-induced mouse parthenogenesis

when immediate-cleavage embryos activated in vitro were transferred at the pronuclear stage to pseudopregnant recipients (Kaufman & Gardner, 1974). In the induced group anaesthetized 20 h after HCG, two out of five females had no implants, and a total of six implants was observed in the remaining three females. Some of the decidual sites have been examined histologically, and have confirmed the presence of implanting embryos (Fig. 1).

DISCUSSION

The present findings confirm that mouse oocytes show an increased tendency to develop parthenogenetically following ageing in the oviduct. Anaesthetics may, by their overall pharmacological action (Krantz & Carr, 1961; Goodman & Gilman, 1970), produce local conditions within the oviduct which stimulate mouse eggs to develop parthenogenetically, though the stimulus involved remains unclear.

These findings stress the necessity of taking into account the possible consequences of anaesthesia, when operative procedures are to be carried out in the early post-ovulatory period. Probably few, if any, of these parthenogenones are capable of prolonged post-implantation viability (Witkowska, 1973b).

It is unlikely that avertin is the only anaesthetic agent which can induce mouse eggs to develop parthenogenetically beyond implantation. While ether anaesthesia was capable of initiating parthenogenetic development (Braden & Austin, 1954a), no activation was observed when eggs were isolated from control oviducts of mice anaesthetized with nembutal (Tarkowski et al. 1970; Witkowska, 1973a), though anaesthesia probably took place during the period when eggs would have been refractory to stimulation by any anaesthetic agent.

While several of the techniques so far described produce limited post-implantation development, oocyte activation by avertin anaesthesia is probably the simplest method of inducing mouse parthenogenesis.

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REFERENCES


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