REDUCTION OF THE OVULATION RATE OF THE MOUSE BY TREATMENT WITH SMALL DOSES OF PREGNANT MARES’ SERUM GONADOTROPHIN

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Summary. The ovulation rate of the mouse was reduced by twice daily injections of small doses of pregnant mares’ serum gonadotrophin (PMSG). A dose of 0.5 i.u. PMSG per injection had the greatest effect, and reduced the number of eggs shed at oestrus and in response to HCG by 3.5 eggs (26%) and 4.7 eggs (34%), respectively. This effect was greater than that of single doses.

The equilibrium level of PMSG which produced the greatest reduction in the ovulation rate was estimated to be 1.5 i.u.

INTRODUCTION

A study of the number of eggs shed by adult mice in response to treatment with single injections of PMSG and HCG showed that, as the dose of PMSG (given at di-oestrus) was increased from zero, the ovulation rate first declined. Only when the dose of PMSG exceeded 2 i.u. did the response start to increase in the conventional manner (Land, 1965).

This communication presents the results of an examination of the number of eggs shed at oestrus and in response to treatment with HCG following repeated injections of PMSG.

MATERIALS AND METHODS

Nulliparous females of the local Q strain were used for all the experiments. Gestyl (Organon) PMSG and Pregnyl (Organon) HCG were used throughout. All preparations were injected intraperitoneally.

Oestrus was determined by pairing females with fertile males, and examining the vaginas each morning for the presence of a copulatory plug. The error associated with this technique is very small, and over 95% of matings can be detected. The ovulation rate was scored between 09.00 and 12.00 hours as the total number of eggs embedded in cumulus in both oviducts. Mice which did not ovulate were excluded from all calculations.

Individual doses of 0, 0.25, 0.5, 1, 2 or 4 i.u. of PMSG were given to each female according to one of the following schedules, a minimum of ten females being allocated to each treatment.

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Schedule 1. Injections were given twice daily at approximately 09.00 and 21.00 hours. Treatment was started regardless of the stage of the oestrous cycle, continued for 3 days before the females were paired with males, and thereafter for a further period of 10 days, or until oestrus occurred. The ovulation rate of those females which showed oestrus during treatment was scored on the morning a plug was found. Those females which did not mate during treatment were divided into two groups. In one, they were left with males until they mated and their ovulation rate scored. In the other, the females were removed from the males and given 2 i.u. of hCG 15 hr after the last pMSG injection, and their ovulation rate scored the following day.

Schedule 2. Treatment was limited to a single injection, given at di-oestrus, at 17.00 hours (when treatment is known to affect the number of eggs shed in response to hCG). The females were then paired with males and their ovulation rate scored at the next oestrus.

Schedule 3. Females were injected twice daily as in Schedule 1, for 2, 4 or 6 days. Doses of 2 or 4 i.u. hCG were given 15 hr after the last pMSG injection, and the ovulation rate was scored the following day.

RESULTS

The number of eggs shed at oestrus

The mean response to all single doses (Schedule 2) lay between 12·5 and 13·5 eggs, and showed that treatment with a single dose of pMSG did not have a significant effect on the natural ovulation rate of those mice which ovulated.

By contrast, the number of eggs shed at oestrus was reduced by repeated injections of pMSG (Schedule 1). The mean ovulation rates of the different groups are given in Table 1, from which it can be seen that twice daily injections of 0·5 and 1 i.u. of pMSG reduced the ovulation rate by 3·5 eggs (26%).

In addition to the decrease in the ovulation rate of the mice which ovulated, it is also apparent that, as the dose of pMSG increased, the proportion of mice showing oestrus and ovulating at the time of oestrus declined. The low ovulation rates of those mice treated with hCG does, however, indicate that the dip in the dose–response cannot be wholly ascribed to a change in follicular sensitivity to luteinizing hormone.

The number of eggs shed in response to HCG

The dose of hCG used to induce ovulation did not have a consistent effect on the number of eggs shed. The two groups were, therefore, pooled, and the response to the three durations of pMSG treatment is illustrated in Text-fig. 1. It can be seen that there is a large reduction in the number of eggs shed following twice daily treatment with 0·25 or 0·5 i.u. of pMSG, irrespective of the duration of treatment. A dose of 0·5 i.u. pMSG per injection reduced the ovulation rate from 13·7 to a mean of 9·1 eggs (34%).

The proportion of animals which ovulated did not decline until the dose of pMSG reached 4 i.u., eight times the dose which produced the greatest dip in the dose–response curve.
**Table 1**

The mean number of eggs shed by nulliparous females at natural oestrus and in response to HCG during and after twice daily treatment with PMSG

<table>
<thead>
<tr>
<th>Dose of PMSG (i.u.)</th>
<th>No. treated</th>
<th>No. with plugs</th>
<th>No. ovulated</th>
<th>Mean ± S.E.</th>
<th>No. with plugs</th>
<th>No. ovulated</th>
<th>Mean ± S.E.</th>
<th>No. treated</th>
<th>No. ovulated</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>13.3 ± 1.4</td>
<td>2</td>
<td>2</td>
<td>10.0 ± 1.9</td>
<td>1</td>
<td>1</td>
<td>3.0 ± 2.0</td>
</tr>
<tr>
<td>1</td>
<td>13</td>
<td>13</td>
<td>6</td>
<td>12.2 ± 2.0</td>
<td>5</td>
<td>4</td>
<td>12.3 ± 1.3</td>
<td>2</td>
<td>2</td>
<td>5.0 ± 1.4</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>11</td>
<td>5</td>
<td>9.8 ± 2.9</td>
<td>6</td>
<td>6</td>
<td>11.8 ± 1.1</td>
<td>3</td>
<td>2</td>
<td>6.0 ± 1.4</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>7</td>
<td>6</td>
<td>13.7 ± 2.0</td>
<td>5</td>
<td>5</td>
<td>11.4 ± 1.2</td>
<td>2</td>
<td>0</td>
<td>—</td>
</tr>
</tbody>
</table>

The standard errors were obtained from the variance pooled within groups.
The above experiments show that repeated injection of \textit{pmsg} can lead to a reduction in the number of eggs shed at both natural oestrus and in response to \textit{hcg}. In both cases, the maximum reduction in the ovulation rate was of a similar magnitude (approximately 30\%) and was caused by twice daily injections of 0.5 i.u. By contrast with the effects of a single dose of \textit{pmsg} on the number of eggs shed following treatment with \textit{hcg} (Land, 1965), single doses of \textit{pmsg} did not affect the number of eggs shed at natural oestrus. This failure of a single dose to reduce the natural ovulation rate may be related to the fact that this is the only situation where the time of ovulation is not determined by the treatment schedule. In all other situations, ovulation took place either during \textit{pmsg} treatment or at a fixed time after treatment. The effects of a single dose of \textit{pmsg} may, therefore, be eliminated before the occurrence of oestrus.

The results of these experiments do not provide any further information about the pathways which lead to the reduction in ovulation rate. They do, however, lead to an estimate of the equilibrium level of \textit{pmsg} which most

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig1.png}
\caption{The mean number of eggs shed by nulliparous females at induced oestrus, following twice daily treatment with different doses of \textit{pmsg} for 2 days (-- -- --), 4 days (---) and 6 days (-----), together with their natural ovulation rate. \textit{N} is the natural ovulation rate.}
\end{figure}
Reduced ovulation in the mouse

effectively inhibits the follicle-stimulating system. This level is not only dependent upon the dose of PMSG which has the greatest effect, but also upon the half-life of PMSG following intraperitoneal injection in the mouse, which has not so far been estimated. If, however, the half-life of PMSG in the mouse is similar to that in the rabbit and the rat, where it has been estimated to be 26 to 36 hr (Lamond, 1960) and 26 hr (Parlow, 1960) respectively, twice daily injections of 0.5 i.u. would lead to a relatively constant level of about 1.5 i.u. within 2 to 3 days. The equilibrium level of PMSG which produces the greatest reduction in the ovulation rate of the mouse is, therefore, very similar to that dose which has the greatest effect when administered as a single injection. We can conclude that approximately 1.5 i.u. is that dose of PMSG which has the greatest inhibitory action on the endogenous follicle-stimulating system relative to its own follicle-stimulating activity when injected intraperitoneally into this particular strain of mice.

REFERENCES

