INFLUENCE OF THE HYPOPHYSIS ON PREGNANT MARE'S SERUM GONADOTROPHIN-INDUCED OVULATION IN IMMATURE RATS

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Summary. The injection of 30 i.u. PMSG in 27-day-old rats produced an ovulatory response which began after 24 to 36 hr and reached a maximum 72 hr after gonadotrophin treatment. There were two increments in the number of ova within this period: between 36 and 48 hr and between 60 and 72 hr. The results of timed experiments involving chlorpromazine blockade or hypophysectomy suggest that the first increment in the number of ova is independent of the pituitary gland but influenced by it. The latter increase seems to be pituitary-dependent since both procedures suppressed it.

INTRODUCTION

Superovulation in the immature rat can be induced by a single injection of PMSG. This effect seems to be due both to a certain intrinsic luteinizing property of PMSG and to its ability to cause release of endogenous ovulating hormone (Williams, 1945a, b).

Strauss & Meyer (1962) have given evidence that ovulation in 32-day-old rats treated with 8 i.u. PMSG depends upon the release of pituitary ovulating hormone during a critical period between 14.00 and 16.00 hours of the 2nd day after PMSG injection. The number of animals which ovulated following hypophysectomy which had been performed at various times before ovulation indicated that the pituitary was essential only until the late afternoon before ovulation. This was confirmed by Quinn & Zarrow (1964) in 24-day-old rats treated with 30 i.u. PMSG (Equinex). Hypophysectomy performed 24, 48 and 52 hr after PMSG completely suppressed ovulation. After 56 hr, ovulation occurred in 38.5%, and after 60 hr, in 87.5%, of the rats. These authors concluded that the length of time required for 30 i.u. PMSG to bring about the release of LH in the 24-day-old immature rat was approximately 56 hr as determined by hypophysectomy, and between 56 and 60 hr as determined by blockade with drugs.

We have produced superovulation in 27-day-old immature rats injected with 30 i.u. PMSG. This treatment is effective in almost 100% of the rats. Most of the ova recovered from the oviducts 72 to 76 hours after PMSG injection were in cumulus. However, some of them were denuded, and even fragmented,

suggesting that the ova had been released from the ovaries at different times, rather than in a single discharge as is generally supposed to occur. As a consequence of these observations, we decided to study in greater detail the process of superovulation in Sprague-Dawley rats injected with 30 i.u. PMSG under the conditions standardized in our laboratory.

MATERIALS AND METHODS

Sprague-Dawley rats bred in our laboratory were kept under controlled lighting conditions in which the light was turned on from 07.00 to 21.00 hours. Unrestricted supplies of pelleted rat food and water were available.

When the rats were 27 days old, they were injected subcutaneously between 09.00 and 10.00 hours (zero hour of the experiments) with 10 or 30 i.u. PMSG (Equinex, Ayerst Laboratories) dissolved in 0.2 ml of 0.9% NaCl solution.

The rats were killed with ether after 24, 36, 48, 60 or 72 hr. Ovaries and uteri were removed and weighed on a torsion balance to the nearest 0.2 mg. Before being weighed, the ovaries were dissected free of adipose tissue and the uteri were freed of mesometrium and luminal fluid. The Fallopian tubes were examined for the presence and number of ova according to the techniques of Rowlands (1942) and Zarrow, Caldwell, Hafez & Pincus (1958).

Chlorpromazine (CP) blocking experiments

A group of rats was injected subcutaneously with CP (Largactil, Laboratorio Colliere) at a dosage of 1 mg/100 g body weight 24 to 28 hr or 48 to 53 hr after the administration of 30 i.u. PMSG. Autopsies were performed 48 or 72 hr after the PMSG had been injected.

Hypophysectomy

Rats injected with 30 i.u. PMSG were hypophysectomized by the transauricular method of Koyama (1962) between 24 and 30 hr, and autopsy was performed 48 hr, after the time of the PMSG injection. Other rats receiving the same treatment were hypophysectomized at 48 hr, and subjected to autopsy 72 hr after PMSG injection.

Another group was hypophysectomized 1 to 24 hr before the injection of 30 i.u. PMSG and autopsy was performed 48 or 72 hr after gonadotrophin treatment. The completeness of hypophysectomy was checked by direct inspection of the sella turcica region and all rats having pituitary fragments were excluded. Rats subjected to sham hypophysectomy served as controls.

After the surgical procedures, the animals were injected with a single dose of 80 μ g 9- α -fluoro-16- β -methylprednisolone (Cidoten, Schering Corporation), and their drinking water was replaced by a solution of 5% glucose.

RESULTS

The number of ova found at various times after the injection of 30 i.u. PMSG is shown in Text-fig. 1. Some ovulations occurred as early as 24 hr after PMSG. By 36 hr, ovulation had occurred in 81% of the rats with an average of 3.9 ± 0.9

ova/rat. The number of ova increased thereafter, reaching a plateau at 48 hr which was maintained throughout the next 12 hr. However, at 72 hr, the number of ova had increased significantly over the number found at 48 to 60 hr.

The ovulatory response 48 hr after the injection of 10 i.u. PMSG was insignificant, but at 72 hr, as related to zero time, 88% of the rats had ovulated, a mean of 18.8 ± 2.3 ova/rat (Table 3).

TEXT-FIG. 1. Time course of the increase in the number of ova collected from the oviducts of 27-day-old rats at different periods after the injection of 30 i.u. PMSG. The abscissa shows intervals of 1 hr and periods of light and darkness. The upper line of numbers under the abscissa represents hours after PMSG injection, which was performed at 0 hr (10.00 hours).

The effects of CP and hypophysectomy are shown in Table 1. Both procedures, when performed between 24 and 30 hr, failed to reduce the ovulatory response at 48 hr. When these procedures were performed at 48 hr, the second increase in the number of ova, which is observed in normal rats at 72 hr, was suppressed. Chlorpromazine injected at 24 hr did not change the normal ovulatory response observed at 72 hr.

When 30 i.u. PMSG were injected in rats hypophysectomized 1 to 24 hr previously, the number of ova found at 48 and 72 hr as related to PMSG injection was significantly smaller than the control values (Table 1).

The effects of different doses of PMSG upon ovulation at 48 and 72 hr are shown in Table 2.

DISCUSSION

The rats used in these experiments ovulated as early as 24 hr after the injection of 30 i.u. PMSG. This result is not in accordance with the reports of other investigators (Strauss & Meyer, 1962; Quinn & Zarrow, 1964), who have given

TABLE 1
EFFECT OF CHLORPROMAZINE OR HYPOPHYSECTOMY ON THE OVULATORY RESPONSE INDUCED WITH PMSG IN 27-DAY-OLD RATS

Treatment	Time of treatment*	Time* of autopsy	No. of rats	No. of ova/rat (mean $\pm$ S.E.M.)	% rats ovulating
Saline	+24	48	46	14.1 $\pm$ 1.7 (a)	93
Saline	+48	72	40	37.6 $\pm$ 4.2 (b)	95
Chlorpromazine 1 mg $\times$ 100 g body wt	+24	48	6	14.3 $\pm$ 6.1 (c)	100
	+28	48	6	9.5 $\pm$ 4.8 (d)	83
	+24	72	10	35.6 $\pm$ 5.5 (e)	100
	+48 to +53	72	40	11.5 $\pm$ 2.3 (f)	80
	-24 to -1	48	6	3.7 $\pm$ 0.8 (g)	100
Hypophysectomy	-24 to -1	48	8	25.5 $\pm$ 3.2 (h)	100
Hypophysectomy	-24 to -1	72	9	1.1 $\pm$ 0.5 (i)	55
Sham operation	-24 to -1	72	11	27.9 $\pm$ 4.5 (j)	90
Hypophysectomy	+24 to +25	48	13	9.7 $\pm$ 3.3 (k)	85
Sham operation	+24 to +25	48	8	10.6 $\pm$ 2.7 (l)	87
Hypophysectomy	+28 to +30	48	10	14.2 $\pm$ 3.1 (m)	90
Sham operation	+28 to +30	48	13	8.9 $\pm$ 2.5 (n)	100
Hypophysectomy	+48	72	9	14.7 $\pm$ 3.8 (o)	100
Sham operation	+48	72	8	34.4 $\pm$ 7.4 (p)	100

Comparison between the number of ova found in the different groups gave the following values of *P* in the Student's test: *P* < 0.001, a versus b; g versus h; i versus j; *P* < 0.01, e versus f; *P* < 0.05, o versus p.

* Time is given in hours after (+) or hours preceding (-) injection of 30 i.u. PMSG (Groups g, h, i, j).

TABLE 2
OVULATORY RESPONSE OF 27-DAY-OLD RATS TO DIFFERENT DOSES OF PMSG

PMSG (i.u.)	Autopsy (hr after PMSG)	No. of rats	No. of ova/rat (mean $\pm$ S.E.M.)	% rats ovulating
5	72	9	8.2 $\pm$ 0.2	100
10	72	17	18.8 $\pm$ 2.3	88
30	72	53	31.4 $\pm$ 2.6	100
10	48	11	0.5 $\pm$ 0.2	27
30	48	46	14.1 $\pm$ 1.7	93

The responses to each dose of PMSG differ significantly in both periods of observations (*P* values in Student's test < 0.001).

evidence that, for ovulation to occur in immature rats following PMSG injection, the release of endogenous LH must take place approximately 56 hr later. This difference may be due to the age of the rats at the time of the gonadotrophin injection, since most of the earlier reports deal with 20- to 24-day-old rats, whereas ours were 27 days old. At this latter age, the rat shows the maximum ovulatory response to PMSG (Zarrow & Quinn, 1963).

Zarrow & Gallo (1969) mentioned the occurrence of ovulation 48 hr after the injection of 30 i.u. PMSG in 30-day-old rats. The response, however, was smaller than that reported here. In two of their experiments, only 9% and 12% of the rats had ovulated at that time and the mean number of ova/rat was 1.5 ± 1.4 and 1.8 ± 1.8 , respectively.

The different strain of the rats may be another source of variation since our rats were Sprague-Dawley and most of the other reports refer to Purdue-Wistar or Holtzman strains. According to Zarrow & Quinn (1963), the last two strains give different responses at 48 hr to the same dose of PMSG.

In our experiments, the number of ova recovered from the oviducts increased after the second and third periods of darkness following PMSG treatment. The first increase was not modified by chlorpromazine treatment or by hypophysectomy performed between 24 and 28 hr after PMSG, whereas both procedures performed at 48 hr suppressed the second increase, normally observed at 72 hr.

TABLE 3

INFLUENCE OF THE PITUITARY ON THE OVARIAN, UTERINE AND OVULATORY RESPONSE OBTAINED IN 27-DAY-OLD RATS 48 HR AFTER THE INJECTION OF PMSG

<i>Time of hypophysectomy</i>	<i>No. of rats</i>	<i>Body weight (g $\pm$ S.E.M.)</i>	<i>Weight of ovaries (mg $\pm$ S.E.M.)</i>	<i>Weight of uterus (mg $\pm$ S.E.M.)</i>	<i>No. of ova/rat (Mean $\pm$ S.E.M.)</i>	<i>% rats ovulating</i>
-24 to -1	6	57.0 ± 6.8	36.03 ± 4.9	40.1 ± 3.0	3.7 ± 0.8	100
+28 to +30	10	59.0 ± 3.2	$60.1 \pm 2.7^{**}$	$62.1 \pm 6.1^*$	14.2 ± 3.1	90

PMSG (30 i.u.) was injected at 10.00 hours. - = hours before PMSG; + = hours after PMSG.

* $P < 0.01$; ** $P < 0.001$.

These results suggest that the ovulatory response to PMSG has two different components: the first one occurs within the first 48 hr and is independent of the pituitary gland, while the second occurs between 60 and 72 hr and is pituitary-dependent.

This finding does not indicate that the pituitary has no influence on the first stage of induced ovulation. The ovulatory response was actually greater in those rats with intact pituitaries during the first 24 hr after PMSG injection than in those which had been previously hypophysectomized. In the former group, the weights of the uteri and ovaries were also greater, which suggests a response to some endogenous gonadotrophins or the participation of other hormones of the pituitary (Table 3).

Ovulation occurring in rats hypophysectomized before gonadotrophin injection must therefore be due to the action of PMSG alone, which is known to possess some weak luteinizing activity. This gonadotrophin has a half-life of 26 hr (Parlow & Ward, 1961). During the first part of this interval, it may stimulate the growth of the ovarian follicles, and the level remaining in the blood 24 to 30 hr after the injection of a large dose may be sufficient to induce ovulation. The low ovulatory response evoked by 10 i.u. PMSG at 48 hr as compared to 30 i.u. (Table 2) is consistent with this concept. With the smaller dose,

the gonadotrophin level remaining after 24 to 30 hr may be subovulatory, and ovulation will occur only after the secretion of LH. This may explain why hypophysectomy, performed 52 hr after the injection of 8 i.u. PMSG in 32-day-old rats, suppressed the ovulatory response to this hormone (Strauss & Meyer, 1962).

Rowlands & Williams (1942) have reported the induction of ovulation in hypophysectomized rats by the injection of two doses of 40 i.u. PMSG given at an interval of 7 days, but not with a single dose. The explanation of this difference may be the fact that their experiments were performed with rats hypophysectomized 5 days previously, so that their ovaries had become atrophic when the gonadotrophin was injected. Since our rats were hypophysectomized shortly before PMSG injection, their ovaries probably contained normally developing follicles at the time when the gonadotrophin began to act.

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