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journal or publication title	Tohoku journal of agricultural research
volume	20
number	4
page range	188-192
year	1970-03-30
URL	http://hdl.handle.net/10097/29581

Tohoku Journal of Agricultural Research
Vol. 20, No. 4, Dec. 1969
Printed in Japan

A Further Evidence for the Release of Ovulating Hormone in the Immature Rats Treated with HCG Alone.

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(Received September 29, 1969)

Summary

The present experiment was undertaken to obtain the further information on the mechanism of ovulation induced by HCG alone in the immature rats.

It was confirmed that if the release of LH is blocked by Nembutal between 52 and 54 hrs after HCG treatment, it will occur by delay of 24 hrs e.g., between 73–76 hrs after HCG. It was also demonstrated that the potency of pituitary ovulating hormone decreased clearly in HCG-treated immature rats, which examined at 72 hrs after HCG and ovulated, although the magnitude of this decreased was very small (12.5%) and furthermore, that the decrease in pituitary ovulating hormone is prevented by administration of Nembutal at 52 hrs after HCG.

The mechanisms for the induction by HCG alone were discussed.

In the previous papers (1,2), it has been demonstrated that spontaneous ovulation, which occurrs at 72 hrs following a single administration of HCG in the immature rats from 24 to 31 days old, is inhibited by the treatment of Nembutal (40 mg/kg body weight) which had been injected at 52 hrs after HCG treatment and that ovulation by HCG alone was associated with the release of luteinizing hormone from the animal's own pituitary.

In order to obtain the further information on the mechanism of ovulation by HCG alone, the present experiment was attempted to estimate the alteration in the content of pituitary hormone in the immature rats treated with HCG.

Materials and Methods

Experimental animals: (A) Donor: Immature Wister strains of female rats, delivered from animals in our own closed coloney were used. The immature rats were 25 days old at beginning of the treatment and they were kept in air conditioned room, whose temperature was 25±1.0°C with 12 hrs light and 12 hrs of darkness.

(B) *Recipient*: Mature virgin female rats of Wister strains, weighing from 190 to 230 gram were used for the assay of ovulating hormone in pituitary of immature rats treated with HCG. Vaginal smears were taken daily for at least two consecutive cycles and rats that had reguraly 4-days cycles was chosen for the assay of the hormone. They were also kept in conditioned room.

Procedures: (A) Donor rats of 25 days old were treated with 20 IU of HCG as reported previously (1) and the animals were injected subcutaneously with Nembutal after 52 hrs of HCG treatment (40 mg/kg body weight). At 72 hrs after HCG administration, the animals were autopsied, and the pituitary, ovaries and oviducts were excised immediately. The removed pituitary was separated from posterior lobe and the weight was recorded. Anterior lobe of each animal was used for the assay of ovulatory potency. Evidence of ovulation was ascertained by the presence of ova in the tube, using the method reported by authors previously (1).

(B) Assay of potency of pituitary ovulatory hormone in the donor rats: The potential of pituitary ovulating hormone in the HCG-treated immature rats was estimated by a method employing ovulation in adult cyclic rats (recipient) at late diestrus as the end point.

The gland of donor rats homogenized with 0.5 ml of physiological saline in a glass tube. 0.5 ml of homogenated gland equivalent to 1/4 or 1/8 of donor rats pituitary was injected intravenously to the recipient rats which had been selected for the assay between 1.00 and 2.00 PM of late diestrus. The injection was carried out under the light ether anesthetization. On the following day, between 9.30 and 10.30 A.M., the recipient rats were killed and examined for the tubal ova.

Results

Time of ovulation in the rats inhibited by Nembutal:

The results are shown in Table 1. At 72 hrs after HCG treatment, ovulation was inhibited by administering Nembutal at 52 hr after HCG. Ovulation occurred in only 3 of 11 rats given this treatment. It was also found that in all rats, in which ovulation inhibited, the uterus was filled with fluid as in the adult rat at

TABLE 1. *Effect of Nembutal on the ovulation time in the immature rats treated with HCG alone.*

Autopsy (Hrs. after HCG) injection	No. of animals	No. of ovulatory animals	Ovulation		Position of ova
			%	No. of ova	
72	11	3	27	4.5±2.3	Ampulla 3/3
96	13	12	97	6.9±1.9	〃 10/12*

Notes:

All rats were administered by 40 mg/kg of Nembutal after 52 hrs of HCG injection (20 IU).

* 2 of 12 rats ovulated had ova in middle of tube, indicating ovulation might be occurred 72 hrs after HCG administration.

proestrus. However, in 96 hrs group, 12 of 13 rats which had been treated with Nembutal 52 hrs later ovulated and 10 of them had a mean of 6.9 eggs located in the ampulla. In 2 of 12 animals ovulated, the ova were found in middle of tube, indicating that ovulation occurred within 72 hrs after HCG treatment.

One of 13 rats killed at 96 hr later was same as proestrus in the adult rat.

Potency of ovulating hormone in donor pituitary:

There was no difference in ovulating potency of pituitary homogenates adjusted equivalent to 1/4 of the gland between rat treated with Nembutal and control animals (Table 2).

TABLE 2. *Ovulatory response of recipient rats to homogenates of donor rat pituitaries*

	Time of removal of pituitary in donor rat Age: (day) A.M	Dose of pitutiary injected	No. of recipient rats	Ovulation		No. of ova (M. ±S.D.)
				No. of recipients rats ovulated	%	
Control (Saline) (ovulated)	28 : 11 : 00	1/4	9	8	90.0	5.0±2.04
		1/8	17	6	35.2	1.6±0.87*
Nembutal (non-ovulated)	28 : 11 : 00	1/4	7	7	100.0	7.4±1.95
		1/8	17	14	82.4	4.5±2.70**

* $P<0.05$ ** $P<0.01$

But, ovulating response of recipient rats to the homogenates equivalent to 1/8 of a pituitary of the control rats decreased markedly in compared to the results obtained in the Nembutal treated-animals. In the group injected by the equivalent to 1/8 pituitary of the Nembutal treated and control rats, the proportions of recipient rats ovulated were 82.4% and 35.2%, respectively. The data indicate that the discharge of the ovulating hormone from pituitary did not occur in the rats administered by Nembutal at 52 hrs after HCG treatment.

In recipient rats injected with the homogenates equivalent to 1/4 of pituitary which originated from Nembutal-treated donor, number of shed ova was many more than that of donor rats of control ($P<0.05$). Especially, in the groups treated with 1/8 of pituitary, there was a significant differences in the number of shed ova between the Nembutal-treated and control rats ($p<0.01$).

Discussion

It has been established that when Nembutal or any of the medium-to long-acting barbiturates is givn to proestrus (4 day cyclic) rats shortly before 2:00 PM ovulation is prevented, while the administration at 4:00 PM does not usually interfere with it.

It is also well known that when the release of LH is blocked on the day of

proestrus, it will occur on the following day between 2:00 PM and 4:00 PM, unless blocked once more (3).

It was shown by Zarrow & Quinn (4) and McCormack & Meyer (5) that ovulation in the PMS-treated immature rat is dependent on the release of ovulating hormone from the pituitary gland during the afternoon of the day preceeding ovulation, and also demonstrated that hypophysectomy later than 56 hrs after PMS treatment did not interfere with subsequent ovulation, but earlier remove of the gland would results in inhibition of ovulation.

A previous paper (2) has shown that Nembutal treatment performed by 52 hr later of HCG injection resulted in inhibition of LH-release in the HCG treated immature rats examined 72 hr later.

In the present experiment, it was found that ovulation in the immature rats, which had been injected with 20 IU HCG and followed by Nembutal treatment 52 hr after HCG, occurred by 96 hrs after HCG treatment.

Therefore, it is considered that Nembutal may acts on the neural mechanism of the LH-release from pituitary in the HCG treated immature rats by same way as well as in adult rats. It was also clear that if the release of LH was blocked by Nembutal between 52 and 54 hrs after HCG treatment, it will occur by delay of 24 hr, e.g. between 76–78 hrs after HCG treatment.

Studies on the potency of ovulating hormone of pituitary in the adult or PMS-treated immature rats were undertaken by several workers (6,7). In a study of pituitary LH throughout the estrus cycle, Schwartz & Bartosik (8) reported that a decrease, which was not significant statistically, during the day of proestrus, but the significant decrease occurred between the morning of proestrus and the morning of estrus. It has been also reported that the significant decrease in pituitary LH content occurred on day between 1:30-2:00 PM after PMS in the immature rats treated with PMS, although the magnitude of this decrease was relatively small (32%) and amounted to only about several μg (1.8–3.4 μg) of LH (7). While it has been demonstrated that pituitary content of LH decreased abruptly at the time of puberty (day of vaginal opening) to a half or a third of its prepuberal level, whether puberty was natural or estrogen-induced (9).

In this experiment, it was confirmed that the potency of pituitary ovulating hormone declines clearly in HCG-treated immature rats, which examined at 72 hrs after HCG and ovulated, although the magnitude of this decline was very small (12.5%), and furthermore, that the decline in pitutary ovulating hormone is prevented by administration of Nembutal at 52 hrs after HCG treatment.

Therefore, it is clear, that the induction of spontaneous ovulation following HCG administration to the intact immature rat is due to release of luteinizing hormone from the animal's own pituitary.

In the previous paper, authors reported that the low dose (5 IU) of HCG resulted in the ovulation in the all immature rats.

Hashimoto et al (10) and Uchida et al (11) have been made the studies on the secretory rate of progestin from the ovary during the estrus cycle in 4 day cyclic rats illuminated 12 hrs a day and confirmed that the progestin secretion increased twice a cycle, one in the evening of proestrus and another during early diestrus.

They also demonstrated that a marked preovulatory rise of progesterone secretion depended upon pituitary LH discharge and a slight increase in both progestin secretion at early diestrus was not dependent on the pituitary functions.

It is possible that the induction of ovulation by HCG alone must be associated primaly with the release of endogenous FSH from the animal's own pituitary. That is, LH-like activity of HCG would primarily result in ovulation or luteinization, acting on some follicles which are responsive to HCG and which produce progestins and the steroids which may in turn stimulate the release of endogenous FSH.

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