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The Potency of the Pituitary Ovulating Hormone in Immature Rats

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Summary

The content of ovulating hormone was determined from 15 days till puberty in immature female rats. The ovulating hormone was observed by the induction of ovulation in adult rats of late diestrus stage. The injection of the suspension from immature pituitary homogenate to recipients (adult rats) was made subcutaneously and intravenously.

In the case of subcutaneous injection, the positive ovulation rate indicated by the pituitary gland in the immature rats was 2/15, 1/7, 7/12, 13/15, 7/8, 9/12, 8/20, 8/14 at 15, 17, 19, 21, 23, 25, 29, 31 days of age respectively. At about 35 days, the positive rate was 5/8, 8/8, 8/14, 8/13, 0/12 at the time of pre-vaginal opening—non-activated uterus, pre-vaginal opening—activated uterus, vaginal opening—non-activated uterus, vaginal opening—activated uterus and vaginal opening—first ovulation, respectively. With intravenous injection, the positive rate was 9/10 for 1/4 gland, 1/9 for 1/8 gland at 15 days. Afterwards, the rate of ovulation was a high 10/11, 9/10, 7/12, 5/6 for 1/8 gland at 21, 29, 35 (pre-vaginal opening—non-activated uterus), 35 (pre-vaginal opening—activated uterus) days respectively in spite of a low of 2/8, 2/7, 1/8, 0/2 for 1/12 glands. At the time of the first ovulation, the rate of ovulation was 4/7 for 1 gland and 0/9 for 1/2 gland. It is suggested that the content of ovulating hormone in immature female rats increases suddenly at about 21 days of age and that the elevated level continues till just before the first ovulation occurs.

We have previously studied the relation between ovarian responsibility to gonadotropin and the onset of puberty (1). The object of the present investigation was to obtain the relationship between the potency of pitutiary gonadotropin and the onset of puberty.

The estimation of the content of pitutiary gonadotropin in immature female rats have been performed by many authors (2-11). Especially concerning pitutiary LH, the alteration of the content on and around puberty has been surveyed to know its relation with the onset of puberty by using the ovarian ascorbic depletion method (6-9, 11). However, pitutiary gonadotropin of immature

rats has not yet been determined as the ovulating hormone which may directly induce ovulation.

Eto, Toyoda and Hoshi had surveyed pituitary gonadotoropic content in adult female rats by the induction of ovulation as an index of the ovulating hormone (12–14). The determination of pituitary ovulating hormone was made according to this method.

Materials and Methods

Immature female rats of the Wistar strain were used in this experiment. The litter size of the new born rats was adjusted to 8 on the next day after parturition. All rats were weaned at 21 days. Afterwards, they were fed with a stock diet and water ad lib. The room was kept at a temperature of 25°C. The light was automatically turned on at 6:00 AM and off at 6:00 PM. The estimation of ovulating hormone was made in conformity to the method of Imamichi et al (15) and Eto et al (12–14). Virgin mature rats of the Wistar strain which indicated regular 4 day cycles were used as the recipients. Anterior pitiutary suspension was injected subcutaneously or intravenously in rats of late diestrus stage at 4:00–6:00 PM. Tubal ova were checked under a dissecting microscope at 20–24 hr. after injection.

This experiment was divided into the subcutaneous and intravenous injection series.

Subcutaneous Injection

Donor animals were autopsied at ages ranging from 15 to 31 days and at near 35 days of pre-vaginal opening or vaginal opening. Concerning those near 35 days; rats were divided into 5 groups i.e.. a) pre-vaginal opening—non-activated uterus, b) pre-vaginal opening—activated uterus, c) vaginal opening—non-activated uterus, d) vaginal opening—activated uterus and e) vaginal opening—first ovulation. After the removal of the anterior pituitary, the gland was homogenizied with cold water and suspended in 0.4 ml saline water.

Intravenous Injection

The pituitary gland was taken out at the ages of 15, 21, 29 and 35 days (i, pre-vaginal opening—non-activated uterus, ii, pre-vaginal opening—activated uterus) and of the first ovulation at vaginal opening. The gland was suspended in 0.5 ml saline solution after homogenization. The gland was given to the recipient by injecting into the right femoral vein under ether anesthesia.

Results

Subcutaneous Injection

There was a gradual increase in the weight of body and pituitary gland from

TABLE 1. *Ovulating Potency of Pituitary in Immature Rats (Recipients were injected subcutaneously).*

Age of days	No. of rats	Body weight (g)	Pituitary weight (mg)	No. of Pituitary injected	Rate of ovulation	No. of ova	Body weight of recipients
15	15	28.5±2.4*	1.6±0.6*	1	2/15	6.5±3.5*	219.7±22.2*
17	7	32.1±3.0	2.0±0.7	1	1/7	10.0±0.0	245.1±19.2
19	12	39.2±3.2	2.5±0.5	1	7/12	8.4±1.4	232.7±25.8
21	15	48.2±5.0	2.5±0.5	1	13/15	7.1±3.5	212.2±20.8
23	8	55.3±2.4	3.0±0.0	1	7/8	8.3±2.7	245.1±20.4
25	12	63.4+4.5	2.8±1.1	1	9/12	10.0±3.0	234.2±21.5
29	20	75.8±8.1	3.6±0.7	1	8/20	7.6±3.2	229.6±23.6
31	14	79.0±6.7	3.6±1.1	1	8/14	7.9±2.8	234.4±35.2
15	8	27.0±4.2	1.1±0.4	2	3/4	7.7±4.8	210.0±13.1
29	10	72.5±5.1	3.7±0.6	2	5/5	11.0±2.0	229.6±14.8
21	3	47.7±4.0	3.7±0.6	1/2	0/6		209.3±25.8
29	3	66.7±9.5	3.5±0.5	1/2	0/5		211.5±33.8

* Mean ±S.D.

15 to on and around the vaginal opening time (Table 1). The rate of ovulation for 1 gland of pituitary was 13 to 14 per cent positive at 15 to 17 days (Table 1). The rate of ovulation increased rapidly to 58 per cent at 19 days. Afterwards, the positive rate remained at a high level (more than 50 per cent) except 40 per cent at 29 days. When the rate of ovulation was compared by χ^2 test, a significant difference was seen between 15 and 19 days ($P<0.05$), and between 15, 17 and 21 days ($P<0.01$). The rate of ovulation at 21 days which indicated a high positive count had no signfiicant difference compared with the subsequent ovulation rate from 23 to 31 days except at 29 days ($P<0.05$). When 2 glands of pituitary were treated, the rate of ovulation at days which indicated a lower rate for 1 gland showed a higher positive count (Table 1). In the case of diluted solution to 1/2

TABLE 2. *Ovulating Potency of Pituitary of Rats at on and around Vaginal Opening*

	Age of days	No. of rats	Body weight (g)	Pituitary weight (mg)
a) Pre-vaginal opening —non-activated uterus	34.8±1.8*	13	95.6± 4.4*	3.5±0.7*
b) Pre-vaginal opening —activated uterus	34.9±1.4	8	104.8± 7.7	4.6±1.4
c) Vaginal opening —non-activated uterus	34.5±2.0	8	101.3± 7.2	4.0±1.1
d) Vaginal opening —activated uterus	32.0±0.7	5	104.8±10.0	5.4±0.5
e) Vaginal opening —first ovulation	35.6±2.5	12	104.1±10.2	5.3±0.8

* Mean±S.D.

gland, ovulation was not seen at 21 days which indicated a high positive count for 1 gland. There was also no ovulation in 29 days. Concerning the groups of pre-vaginal and vaginal opening, group of a), b), c) and d) showed a high positive count of more than 50 per cent regardless of the occurence of vaginal opening and the presence of uterine fluid (Table 2). There was not a significant difference in the rate of ovulation among these 4 groups, and the rate of all the groups were not different comparing with those of 21 days. Group e) of the first ovulation at vaginal opening whose ova were seen in ampulla did not show any ovulation for 1 gland. There was a significant difference in the rate of ovulation when e) was compared with the above 4 groups (group a), b), c): $P<0.01$, group d): $P<0.05$). When the number of ova in the positve cases was compared from 15 to 31 days, there was no difference. Also no difference was seen when comparing the 4 points on and around the vaginal opening in positive cases.

Intravenous Injection

Because the ovulation response to the intranveous test was more sensitive than the subcutaneous (14, 15), intravenous injections were used in order to detect further details of the alteration of the pituitary ovulating hormone. The weights of the body and the pituitary gland in this series were not so different from those in the subcutaneous test at the age of 15, 21, 29 and 35 days respectively (Table 3). The positive rates of ovulation were 90 per cent for 1/4 gland, 11 per cent for 1/8 gland at 15 days; and 91 per cent for 1/8 gland, 25 per cent for 1/12 gland at 21 days. The minimum effective dose for more than 50 per cent positive ovulation was 1/4 gland in 15 days and 1/8 dose in 21 days. Accordingly, the ovulating hormone per 1 pituitary gland seems to increase to about two fold between 15 to 21 days. From the result of tests at 29 days and 35 days which includes both types of activated and non-activated uterus, the minimum effective dose for ovulation was not different from that of 21 days. However, 1 gland was necessary for

(Recipients were injected subcutaneously)

Ovarian weight (mg)	Uterine weight (mg)	No. of pituitary injected	Rate of ovulation	No. of ova	Body weight of recipients
17.4±3.5*	63.2±19.6*	1	8/12	8.8±3.5*	228.3±17.8*
21.4±4.4	182.1±45.1	1	8/8	5.0±2.9	242.1±32.1
18.3±2.5	83.3±12.8	1	5/8	4.8±3.0	236.1±24.7
24.2±4.2	234.4±17.6	1	3/5	7.3±1.2	225.0±14.6
32.7±3.9	146.6±17.4	1	0/12	—	235.8±25.0

TABLE 3. *Ovulating Potency of Pituitary in Immature Rats*

Age of days	No. of rats	Body weight (g)	Pituitary weiuht (mg)	1
15	10	27.9± 2.6*	1.2±0.4*	
21	20	47.3± 3.4	2.2±0.7	
29	11	76.6± 6.7	3.2±0.6	
35 (pre-vaginal opening—non-activated uterus)	16	101.1± 7.5	4.1±0.7	
35 (pre-vaginal opening—activated uterus)	6	106.2± 8.4	4.8±0.8	
35.6±5.4 (first ovulation at vaginal opening)	16	102.6±12.2	4.9±0.5	4/7 (5.0±2.6)

* Mean±S.D.

the minimum dose in rats of first ovulation at vaginal opening. There was no occurence of ovulation by treatment of 1/2 gland.

Discussion

From the results of the above subcutaneous and intravenous injections, it was proved that the ovulating potency of pituitary gland for using a constant dose of gland in the infantile period for about 15 days shows an abrupt increase at about 21 days and the elvated level continues till the period just before the first ovulation occurs. The slightly low potency at 29 days shown with subcutaneous injections seems to be negligible from the results of the intravenous test. The gradually increasing weight of the pituitary gland seems to be unrelated directly with the potency of the hormone.

The sudden decrease of LH at puberty had been reproted by using the ovarian ascrobic acid depletion method (8, 9, 11). The results in this study also support the theory of a decreased gonadotropin at puberty as an ovulating hormone. However, it is known from our study that the sudden decrease of the hormone does not always occur at the vaginal opening. In addition, ovulation at the vaginal opening can be found in about 50 per cent of all rats (16). Therefore, the estimation of pituitary ovulating hormone should be made by groupings according to the occurrence or nonoccurrence of ovulation. It is interesting that the potency of the pituitary ovulating hormone remains constant from about 21 days to near puberty. There is no evidence that the plasma level of LH appeared before puberty in immature female rats (8, 9). Accordingly, the mechanism of the central nervous system for abrupt release of pituitary ovulating hormone seems to be important concerning the onset of puberty.

(Recipients were injected intravenously)

No. of pituitary injeccted					
1/2	1/4	1/6	1/8	1/10	1/12
Rate of ovulation (no. of ova)					
0/9	9/10 (7.6±4.6) 5/ 5 (8.0±2.2) 3/ 3 (9.3±5.5)	2/4 (3.0±2.8) 4/5 (5.3±1.0) 4/5 (4.5±1.9) 3/4 (3.7±3.0)	1/ 9 (2.0±0.0) 10/11 (7.2±2.5) 9/10 (4.7±2.4) 7/12 (3.7±2.9) 5/ 6 (5.4±2.9)	3/6 (7.3±3.8) 2/3 (3.0±0.0) 2/7 (2.5±2.1) 0/2	2/8 (3.0±0.0) 2/7 (3.0±1.4) 1/8 (1.0±0.0) 0/2

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