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Effect of LH-RH, Gonadotrophins or Sex Hormone Treatment on RNA and DNA Concentration in Hypothalamus, Pituitary, Ovary and Uterus in the Immature Female Rat

M. E. Díaz-Díaz *, J. C. Díaz-Chico and B. N. Díaz-Chico

Departamento de Fisiología y Bioquímica
Colegio Universitario de Las Palmas
Las Palmas de Gran Canaria
Islas Canarias (Spain)

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RNA and DNA concentration was measured in hypothalamus, pituitary, ovary and uterus of immature female rats after treatment with 5 doses of LH-RH, PMSG, HCG, FSH + LH, estradiol benzoate or progesterone. All assayed hormones decreased DNA concentration and increased the [RNA]/[DNA] ratio in their target organs. These findings are interpreted as increases in cell volume and RNA synthesis in target organs after treatment. Gonadotrophins and sex hormones decreased DNA concentration and increased RNA synthesis in hypothalamus and pituitary, which revealed the stimulatory effect of both hormonal groups on the above mentioned organs.

In previous papers (4, 5) we showed that LH-RH increased both RNA and protein synthesis in the immature female rat pituitary *in vitro*.

Here the effect of five doses of LH-RH, gonadotrophins or sex hormones on RNA and DNA concentration in hypothalamus, pituitary, ovary and uterus of immature female rats has been studied *in vivo* in order to verify if a specific hormonal treatment modifies the nucleic acid concentration in its target organ, and if the feed-back effects of sex hormones provokes also hypothalamic or pituitary alterations in nucleic acids.

Materials and Methods

Female Wistar rats, 21-day-old, suckling their mothers until the first injection and then sustained with water and food *ad libitum* until sacrificed, were subjected to cycles of 14 h light and 10 h darkness. Groups of 10 animals were injected subcutaneously during 5 days with a daily single dose of one of the following hor-

* Present address: Departamento de Farmacología, Facultad de Medicina, La Cuesta. Tenerife. Islas Canarias (Spain).

mones*: 10 μg LH-RH (Beckman); 60 *UI* PMSG (Leo); 50 *UI* HCG (Leo); 5 *UI* FSH plus 5.6 *UI* LH (Farma-Lepori); 5 mg Progesterone; 250 μg estradiol benzoate (Schering) or 0.25 ml of saline or corn oil (control groups).

Animals were decapitated 24 h after the last injection, and their organs were rapidly dissected weighed and chilled at —40° C, and stored at this temperature. RNA and DNA were measured according to SCHNEIDER (11). Briefly, the organs were homogenized in 1 ml cold saline, precipitated with 1 ml cold 20 % TCA and centrifuged. The precipitate was washed twice with 95 % ethanol at 50° C. Nucleic acids were extracted from the last precipitate by 10 % TCA. 30 min at 95 ° C and further centrifugation.

Colour reaction for DNA was obtained by 0.5 ml supernatant and 1 ml diphenylamine reagent — 1 g diphenylamine (BDH) in 100 ml acetic acid and 2.75 ml sulphuric acid — and measured at 610 nm. Calf thymus DNA (BDH) was used as standard. Colour reaction for RNA was obtained by 0.5 ml supernatant and 2.5 ml orcinol reagent — 1 g orcinol (BDH), 0.5 g $FeCl_3$ in 80 ml concentrated HCl and 50 ml redistilled water — and measured to 660 nm. Yeast RNA (BDH) was used as standard.

Results are expressed as mg of nucleic acid per 100 mg wet weight tissue (mean ± S.E.M.). They were analyzed for variance according to Snedecor F test, and with controls according to Student t test.

Results and Discussion

Figure 1 shows that PMSG treatment increases RNA and DNA content in the ovary of immature female rats. Tables I and II show the decrease in DNA and RNA concentration in ovary after PMSG treatment. The RNA content in ovary was increased by all the assayed gonadotrophin treatments (fig. 1B). The ratio [RNA]/[DNA] increased after the HCG and FSH + LH treatment, but was not altered by PMSG (table III).

It is possible to infer what occurs to nucleic acids by studying their concentrations in the present results. DNA synthesis takes place only with [DNA] increases. A decrease in [DNA], caused by dilution, can be interpreted as an increase in cell or organ volume.

A [DNA] decrease and an unchanged [RNA]/[DNA] mean that both nucleic acids are diluted in the same proportion,

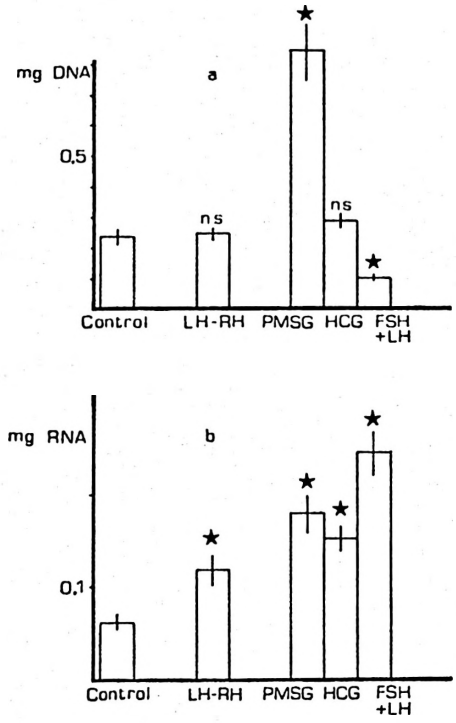

Fig. 1. *Effect of 5 doses of LH-RH, PMSG, HCG or FSH + LH on ovarian content in DNA (a) and RNA (b), expressed in mg/100 mg. of wet weigth.*
n.s. = Non significant differences. ★ = $p > 0.001$.

* Abbreviations:
LH-RH: Luteinizing Hormone-Releasing Hormone.
PMSG: Pregnant mare's serum gonadotrophin.
FSH: Follicle Stimulating Hormone.
LH: Luteinizing Hormone.

Table I. *Effect of 5 doses of LH-RH, PMSG, HCG, FSH+LH, Estradiol or Progesterone on DNA concentration of immature female rat hypothalamus, pituitary, ovary and uterus.* Results (mean ± S.E.M.) in mg DNA/100 mg w.w. tissue. No. of animals in parentheses. Comparisons with controls were effectuated by Student t test.

Treatment	Hypothalamus	Pituitary	Ovary	Uterus
Controls	(10) 0.94±0.06	(10) 7.79±0.72	(9) 2.35±0.19	(10) 6.87±1.05
LH-RH	(7) 0.28±0.04 [a]	(7) 3.55±0.35 [a]	(8) 2.36±0.24	(7) 4.07±0.41 [c]
PMSG	(9) 0.50±0.03 [a]	(9) 3.57±0.20 [a]	(10) 1.51±0.11	(10) 0.80±0.08 [a]
HCG	(10) 0.55±0.05 [a]	(10) 2.50±0.13 [a]	(9) 0.93±0.11 [a]	(8) 0.69±0.09 [a]
FSH+LH	(9) 0.56±0.09 [b]	(7) 1.76±0.24 [a]	(9) 0.36±0.02 [a]	(9) 0.92±0.07 [a]
Estradiol	(7) 0.54±0.05 [a]	(7) 3.21±0.15 [a]	(7) 2.30±0.14	(6) 1.15±0.12 [b]
Progesterone	(8) 0.58±0.04 [a]	(8) 6.06±0.41	(9) 3.66±0.26 [b]	(6) 2.27±0.09 [b]
Analysis of variance				
F ratio	12.24	29.75	<0.01	<0.01
P value	<0.01	<0.01	42.57	23.30

a) Means $p < 0.001$; b) $p < 0.01$; c) $p < 0.05$; otherwise no significant differences with controls appear.

which masks a possible RNA synthesis and, therefore, makes it impossible to interpret it as such. But a [DNA] decrease and a [RNA]/[DNA] increase may be interpreted as RNA synthesis because a greater dilution of DNA over RNA can only take place when the RNA content of the cell increases.

The ovarian nucleic acid decrease after PMSG treatment can be explained by the great ovarian growth observed, which implies an intense cellular division and increases in follicular liquid quantities, masking the DNA and RNA synthesis. HCG does not alter DNA content in ovary but increases RNA synthesis, interpreted as the adjustment of the cells to the intense steroid synthesis accompanying luteinization. FSH + LH treatment decreases the DNA content, probably due to numerous ovulations, revealed by the high number of hemorrhagic follicles

Table II. *Effect of 5 doses of LH-RH, PMSG, HCG, FSH+LH, Estradiol or Progesterone on RNA concentration of immature female rat hypothalamus, pituitary, ovary and uterus.* Results (mean ± S.E.M.) in mg RNA/100 mg w.w. tissue. No. of animals in parentheses. Comparisons with controls were effectuated by Student t test.

Treatment	Hypothalamus	Pituitary	Ovary	Uterus
Control	(9) 0.23±0.01	(9) 1.19±0.10	(9) 0.60±0.06	(9) 1.44±0.22
LH-RH	(7) 0.27±0.01	(6) 1.94±0.27 [c]	(6) 1.08±0.12 [b]	(7) 1.47±0.41
PMSG	(9) 0.24±0.02	(8) 1.03±0.10	(10) 0.32±0.03 [b]	(10) 0.23±0.02 [a]
HCG	(10) 0.23±0.02	(9) 1.13±0.22	(9) 0.51±0.05	(7) 0.48±0.05 [a]
FSH+LH	(8) 1.18±0.06 [a]	(8) 2.16±0.18 [a]	(8) 0.89±0.06	(9) 0.90±0.08
Estradiol	(7) 0.21±0.01	(7) 1.07±0.06	(7) 0.59±0.04 [b]	(7) 0.41±0.03 [b]
Progesterone	(9) 0.23±0.02	(7) 1.65±0.18	(9) 0.68±0.05	(8) 0.56±0.04 [b]
Analysis of variance				
F ratio	172.85	7.40	18.64	9.14
P value	<0.01	<0.01	<0.01	<0.01

a) Indicates $p < 0.001$; b) $p < 0.01$; c) $p < 0.05$; otherwise no significant differences with controls appear.

Table III. *Effect of 5 doses of LH-RH, PMSG, HCG, FSH+LH, Estradiol or Progesterone on (RNA)/(DNA) relation of immature female rat hypothalamus, pituitary, ovary and uterus.*

Results = mean ± S.E.M. No. of experiments in parentheses. Comparisons with controls were performed by Student t test.

Treatment	Hypothalamus	Pituitary	Ovary	Uterus
Control	(9) 0.26±0.01	(9) 0.17±0.01	(9) 0.27±0.02	(9) 0.20±0.02
LH-RH	(7) 1.06±0.11 [a]	(6) 0.56±0.06 [a]	(6) 0.51±0.05 [a]	(7) 0.36±0.03 [a]
PMSG	(9) 0.49±0.02 [a]	(8) 0.29±0.02 [a]	(10) 0.22±0.01	(10) 0.29±0.02
HCG	(10) 0.45±0.05 [b]	(9) 0.45±0.07 [a]	(9) 0.59±0.03 [a]	(7) 0.77±0.03 [a]
FSH+LH	(8) 2.49±0.37 [a]	(7) 1.41±0.23 [a]	(8) 2.51±0.26 [a]	(9) 1.00±0.07 [a]
Estradiol	(7) 0.41±0.04 [b]	(7) 0.33±0.02 [a]	(7) 0.36±0.02 [c]	(6) 0.36±0.02 [a]
Progesterone	(8) 0.41±0.02 [a]	(7) 0.20±0.02 [b]	(9) 0.25±0.02	(8) 0.25±0.02
Analysis of variance				
F ratio	30.21	22.31	70.02	66.16
P value	<0.01	<0.01	<0.01	<0.01

a) Means $p < 0.001$; b) $p < 0.01$; c) $p < 0.05$; otherwise no significant differences with controls appear.

found in them. These findings agree with those described by previous authors (2, 10).

The LH-RH treatment produces [RNA] and [RNA]/[DNA] increases, and [DNA] decrease in pituitary, which implies RNA synthesis and cell volume increase in agreement with our previous results (4, 5). In the ovary, the LH-RH treatment increases [RNA] and [RNA]/[DNA], while [DNA] goes unchanged. This RNA synthesis is due to a gonadotrophin release of the pituitary, and agrees with results by DEBELJUK *et al.* (3) who showed that LH-RH provokes a release of gonadotrophins from the immature female rat pituitary.

Uterine [DNA] and [RNA] decrease after sex hormone treatment to immature female rats. Estradiol increases [RNA]/[DNA], which implies RNA synthesis, in accordance with the results of other authors (12, 13, 15, 16).

All the assayed treatments increased [RNA]/[DNA] and decreased [DNA] in the immature female rat hypothalamus. This RNA synthesis agrees with CALIGARIS *et al.* (1), and other authors (4, 6) who describe increases in hypothalamic metabolism in the presence of estradiol. The pituitary modifications observed after the estradiol treatment are similar to those provoked by the LH-RH treatment. They can be due either to a direct effect of this hormone on the pituitary, or to a potentiation of the LH-RH effect on this gland, as described by SPONA (14).

The gonadotrophin effects on hypothalamus and pituitary glands could be explained as an indirect effect of the sex hormones, whose ovarian production by the immature ovary has been described by the above mentioned mechanism (7, 8). However, OJEDA and RAMÍREZ (9) described a direct action of gonadotrophins on the hypothalamus, with a stimulatory effect by FSH and a negative effect by LH on the immature female rat. The lack of effect on the uterus by LH-RH treatment, implying the absence of stimulation on ovarian sex hormone release, suggests that the RNA synthesis in the hypothalamus after LH-RH treatment is due to a stimulatory action of pituitary gonadotrophins. This hypothesis is also supported by the stimulatory effect of FSH + LH treatment on hypothalamus and pituitary.

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Resumen

Se miden las concentraciones de DNA y RNA en hipotálamo, hipófisis, ovario y útero de ratas hembras inmaduras después del tratamiento con cinco dosis de LH-RH, PMSG, HCG, FSH + LH, benzoato de estradiol o progesterona. Todas las hormonas ensayadas disminuyeron la concentración de DNA y aumentaron la relación [RNA]/[DNA] en sus órganos diana después del tratamiento. Estos resultados son interpretados como aumentos en el volumen celular y en la síntesis de RNA en dichos órganos después del tratamiento. Las gonadotrofinas y las hormonas sexuales disminuyeron la concentración de DNA y aumentaron la síntesis de RNA en hipotálamo e hipófisis.

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