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THE EFFECT OF ICSH-β AND ITS COMBINATION WITH PROLACTIN ON THE MAINTENANCE OF PREGNANCY IN THE RAT

By

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ABSTRACT

In the rat hypophysectomized on day 5 of pregnancy, replacement hormonal treatment daily with 100 μg ICSH or 2 mg prolactin alone was not effective for maintenance of pregnancy, whereas the combination of 50–200 μg ICSH and 2 mg prolactin gave live embryos in 40–90 % of treated animals. In the animal hypophysectomized at more advanced stage of pregnancy, ICSH alone was able to maintain pregnancy. When 400 μg ICSH-β was used instead of 100 μg ICSH in conjunction with 2 mg prolactin, this treatment was also effective for maintenance of pregnancy in the animal hypophysectomized on day 5. If hypophysectomized on day 8, either reconstituted ICHS or ICHS-β alone could maintain pregnancy. ICSH-α was ineffective in the animal hypophysectomized on either day 5 or day 8 of pregnancy. Thus it appears that ICSH-β is the biologically active subunit in the ICSH molecule for maintenance of pregnancy in the rat.

Earlier studies with highly purified pituitary hormones in the mated rats, which have been hypophysectomized on day 1 or 6 of gestation, showed that daily treatments with either prolactin, FSH or ICSH alone were ineffective, whereas the combination of prolactin, FSH and ICSH (*Ahmad et al.* 1969) or that of prolactin and FSH (*Greenwald & Johnson* 1968) were effective for maintenance of pregnancy. In animals which have been hypophysectomized on day 8 or 9 of pregnancy, however, the administration of ICSH alone maintained pregnancy (*Alloiteau & Bouhours* 1965; *Moudgal* 1969).

Table 1.

Maintenance of pregnancy in the mated rats hypophysectomized on Day 5 with subsequent ICSH and prolactin treatment. Animals were autopsied on Day 14 for examination of implantation sites and live embryos.

Group No.	Daily doses of		No. of animals			Implantation sites mean ± SE	Live embryos mean ± SE
	Prolactin mg	ICSH μg	treated	with impl. sites	with live embryos		
1	2	25	10	8	0	8.8 ± 1.5	0
2	2	50	10	10	9	12.0 ± 0.6	8.6 ± 1.4[a)b)]
3	2	100	10	10	4	13.5 ± 0.4	4.8 ± 2.4
4	2	200	10	10	5	10.3 ± 1.1	5.5 ± 2.3
5	0	50	10	0	0	0	0
6	0	100	10	1	0	0.5 ± 0.3	0
7	0	0	10	5	0	4.8 ± 2.0	0
		Hypophysectomized controls with gelatin treatment					
8	0.4 ml 20 %	gelatin alone and autopsy on Day 6	10	–	0	–	–
9	0.4 ml 20 %	gelatin alone and autopsy on Day 7	10	10*	0	10.8 ± 1.3*	–
10	0.4 ml 20 %	gelatin alone and autopsy on Day 8	10	10*	0	11.0 ± 1.4*	–
11	0.4 ml 20 %	gelatin alone and autopsy on Day 14	10	0	0	0	0
		Intact pregnant controls with gelatin treatment					
12	Intact pregnant females treated with 20 % gelatin		10	10	10	12.6 ± 0.2	11.6 ± 0.4[c)]
13	Intact pregnant females without any treatment		10	10	10	11.5 ± 0.6	10.7 ± 0.7

* There were no implantation but uterine swelling caused by decidual reaction due to degenerating eggs.

a) Significant difference by t-test ($t = 6.14$, $P < 0.01$) when compared with group 6.

b) Significant difference by t-test ($t = 6.14$, $P < 0.01$) when compared with group 7.

c) No significant difference by t-test ($t = 1.12$, $P > 0.10$) when compared group 13.

The present studies were stimulated by the recent findings that the β-subunit of ovine ICSH possesses the capability to induce ovulation in the hamster (*Yang et al.* 1972). The following experiments were designed to study the effect of ICSH-β and its combination with prolactin for maintenance of pregnancy in the hypophysectomized rat.

MATERIALS AND METHODS

Primigravid Long-Evans rats weighing 210 to 230 g were mated with fertile males on the afternoon of pro-oestrus. The presence of sperms in the vagina was designated as day 1 of pregnancy. Hypophysectomy was performed on the 5th, 6th or 8th day of pregnancy, by the parapharyngeal approach under ether anaesthesia between 16.00 and 18.00 h. The animals were autopsied on day 14 for inspection of implantation sites and live embryos. Only those embryos which showed heart beat under stereomicroscopical observation were classified as live.

Daily injections of varying doses of ICSH in conjunction with 2 mg prolactin were given from day 5 to 13 immediately after hypophysectomy on day 5 of gestation. Two groups of control animals received either ICSH or prolactin alone for the same period. Another 4 groups of females which have been hypophysectomized on day 5 followed by daily injections of 0.4 ml 20 % gelatin were autopsied either on day 6, 7, 8 or 14 for examination of their implantation sites by histological sections and haematoxylin-eosin staining. Furthermore, 2 groups of intact pregnant females were either injected or not injected with 20 % gelatin from day 5 to 13 for comparison of the effect of hormonal vehicle alone on the pregnancy.

For examination of the effect of ICSH at later stages of gestation, the animals were treated with 25 to 100 μg of the hormone or with prolactin, following hypophysectomy on day 6 or 8.

The efficacy of either of the ISCH subunits α and β (400 μg each) to maintain pregnancy in animals hypophysectomized on day 5 was studied by administration of these preparations in combinations with 2 mg of prolactin. In addition, the subunits or the reassociated ICHS (200 μg) were administered daily to females hypophysectomized on day 8 for studying their effect on the post-implantation stages.

Ovine ICSH was isolated according to the method of *Papkoff et al.* (1965). The ICSH subunits and the reassociated molecule were prepared as outlined by *Sairam et al.* (1972). Ovine prolactin was prepared according to the method of *Li et al.* (1970). All hormone preparations were dissolved in 0.2 ml of distilled water which was made slightly alkaline with dilute sodium hydroxide; this solution was mixed with an equal volume of 20 % aqueous solution of gelatin at 40°C before each injection.

RESULTS

Before studying the effect of the ICSH subunits alone or in combination with prolactin to maintain pregnancy, experiments were performed with native ICSH with a view to standardize the conditions. In animals hypophysectomized

Table 2.

Maintenance of pregnancy in the mated rats hypophysectomized on Day 6 or 8 of gestation followed by ICSH or prolactin treatment alone.

Group No.	Day of hypophys-ectomy	Daily dose		No. of animals			Implantation sites mean ± SE	Live embryos mean ± SE
		ICSH μg	Prolactin mg	treated	with impl. sites	with live embryos		
14	6	100	0	9	5	2	6.8 ± 2.2	1.7 ± 1.4
15	8	25	0	10	8	0	9.0 ± 1.5	0
16	8	50	0	10	10	4	10.2 ± 1.0	3.6 ± 1.7
17	8	100	0	11	11	6	12.4 ± 0.6	6.3 ± 2.1[a]
18	8	0	2	10	6	0	5.5 ± 2.6	0
19	Control; 0.4 ml, 20 % gelatin alone[b]			10	1	0	1.3 ± 1.3	0

a) Significant difference by t-test ($t = 2.85$, $P < 0.02$) when compared with group 19.

b) Hypophysectomy on Day 8.

on day 5 of gestation, treatment with 25 μg ICSH and 2 mg of prolactin was ineffective, but administration of either 50, 100 or 200 μg of ICSH in conjunction with 2 mg prolactin maintained pregnancy in 40 to 90 % of the treated animals (Table 1, groups 1–4). ICSH or prolactin administered alone was only effective for maintenance of few implantation sites but unable to induce live embryos. Following gelatin treatment after hypophysectomy on day 5 in the control groups, uterine swellings induced by decidual reaction to degenerating eggs were formed on day 7 and the uterine swellings retrogressed markedly on day 8 and completely resorbed by day 14 (groups 8–11). Gelatin treatment has shown neither deteriorative nor beneficial effect on the process of implantation and embryonic development in the intact pregnant rats (groups 12 vs. 13).

At the more advanced stage of pregnancy such as on day 6 or 8, 100 μg ICSH alone was effective in maintaining pregnancy in 22 % and 57 % respectively. However, 2 mg prolactin alone maintained moderate number of implantation sites without live embryos. For control, gelatin treatment was not contributory for maintenance of implantation sites (Table 2, groups 14–19).

When the subunits of ICSH (Table 3) were administered instead of the native hormone in combination with prolactin, ICSH-α (400 μg) was ineffective (group 20) whereas 400 μg ICSH-β maintained pregnancy in 47 % of the treated animals (group 21). It may be noted that 400 μg ICSH-β alone was unable to maintain pregnancy in the rat hypophysectomized on day 5 (group 22). As expected, ICSH-α was again ineffective (group 23) in pregnancy maintenance in animals hypophysectomized on day 8 but pregnancy was maintained in 40 % of the animals by 400 μg of ICSH-β (group 24). It can also be seen that 200 μg of reassociated molecule (ICSH-R) prepared *in vitro* by an appropriate combination of the individual subunits maintained pregnancy in over 50 % of the animals (group 25).

DISCUSSION

In agreement with *Ahmad et al.* (1969), the present studies show that prolactin (2 mg) and low doses of ICSH (25 μg or less) failed to maintain pregnancy but higher doses of ICSH (50 μg) with prolactin maintained pregnancy in the majority of the animals hypophysectomized on day 5 (see Table 1). Apparently, FSH did not seem to be essential, as none of the animals were treated with this hormone. This observation differs from that of *Ahmad et al.* (1969) who concluded that prolactin plus ICSH, prolactin plus FSH or ICSH plus FSH could not insure pregnancy maintenance in rats hypophysectomized during early pregnancy. Their failure to maintain pregnancy in animals hypophys-

Table 3.

Maintenance of pregnancy in the hypophysectomized rat by administration of ICSH-R, or ICSH-β alone from Day 8 or by ICSH-β plus prolactin from Day 5.

Group No.	Day of hypophys-ectomy	Treatment	No. of animals			Implantation sites mean ± SE	Live embryos mean ± SE
			treated	with impl. sites	with live embryos		
20	5	400 μg ICSH-α 2 mg prolactin	15	10	0	5.4 ± 1.1	0[a]
21	5	400 μg ICSH-β 2 mg prolactin	15	14	7	9.6 ± 1.1	3.3 ± 1.0
22	5	400 μg ICSH-β	10	1	0	0.7 ± 0.7	0
23	8	400 μg ICSH-α	15	2	0	4.6 ± 2.1	0[b]
24	8	400 μg ICSH-β	10	9	4	10.0 ± 1.2	2.9 ± 1.3
25	8	200 μg ICSH-R	8	7	4	11.5 ± 1.7	5.1 ± 2.1

a) Significant difference by t-test ($t = 3.3$, $P < 0.01$) when compared with group 21.
b) Significant difference by t-test ($t = 2.76$, $P < 0.02$) when compared with group 24.

ectomized on day 5 is probably due to the fact that very low dosages of ICSH (not more than 10 μg) was employed along with prolactin.

Exogenous administration of prolactin is not required for pregnancy maintenance in animals hypophysectomized on day 8 (Table 2, groups 16–17). *Alloiteau & Bouhours* (1965) and *Moudgal* (1969) also made similar observations by administering ICSH in a delaying agent. Evidence is accumulating that at this stage of pregnancy the mammotrophic factor from the rat placenta presumably contributes to luteal maintenance (*Astwood & Greep* 1938; *Averill et al.* 1950; *Ray et al.* 1955).

An important observation of the current studies is that, while the β-subunit of ovine ICSH is capable of maintaining pregnancy when given alone in hypophysectomized rats on day 8, its counterpart (the α-subunit) is inactive in this respect. This suggests that the isolated β-subunit possesses the ability to interact with the target organ (the pregnant ovary) to initiate events that lead to synthesis of progesterone required for pregnancy maintenance. It has been demonstrated in the hamster that ovulation inducing activity of ovine ICSH resides in its β-subunit (*Yang et al.* 1972). According to the suggestion of *Lipner & Greep* (1971), ovulation requires the participation of steroid hormones. Thus, ICHS-β must be capable of stimulating steroid production. Direct proof for this contention must inevitably come from actual measurements of progesterone synthesis from actively secreting corpora lutea. It should be mentioned in this connection that *Gospadarowicz* (1971) reported the requirement of both subunits for the expression of steroidogenic potential of the ICHS molecule. The discrepancy between his results and those of the present study requires further study.

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