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Effect of prolactin on the production of progesterone by mouse ovaries *in vitro*

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Prolactin constitutes part of the luteotrophic complex necessary for the maintenance and secretory activity of the CL in the rat (Evans, Simpson, Lyons & Turpeinen, 1941; Astwood, 1941), mouse (Kovacic, 1964), rabbit (Spies, Hilliard & Sawyer, 1968), hamster (Greenwald & Rothchild, 1968), ferret (Donovan, 1963), pig (du Mesnil du Buisson, 1973), and sheep (Denamur, Martinet & Short, 1973). There is also evidence from in-vitro studies that low concentrations of prolactin are essential for the production of progesterone by preovulatory human granulosa cells (McNatty, Sawers & McNeilly, 1975; McNatty, Bennie, Hunter & McNeilly, 1976) while high concentrations are inhibitory (McNatty *et al.*, 1976). In the present study the effect of various concentrations of prolactin on the production of progesterone by mouse ovaries in organ culture with or without luteal tissue was investigated, and the effects of rat, sheep or human prolactin preparations on ovarian steroidogenesis were compared.

Mice of the Schofield albino strain were given an i.p. injection of 5 i.u. PMSG (Gestyl: Organon Laboratories) at 25 or 28 days of age. Forty hours later the animals were either killed by cervical dislocation (animals without CL) or given an ovulating injection of 2 i.u. HCG (Pregnyl: Organon Laboratories) and killed 24 hr later (animals with CL). The ovaries were removed, cut into fragments and set up in the organ culture system of Neal, Baker, McNatty & Scaramuzzi (1975). The culture was maintained (i) without added hormones; (ii) with prolactin (rat, NIAMD-RP1; ovine, NIH-P-S8; human, Friesen preparation); (iii) with prolactin + ovine FSH (NIH-FSH-S9) + LH (NIH-LH-S18); (iv) ovine FSH + LH (1 μg/ml of each). After 24 hr in culture the medium was removed and stored at −18°C until assayed for progesterone by the method of Neal *et al.* (1975). The ovarian fragments were fixed in Bouin's aqueous fluid and serial sections of the ovaries were examined for the presence or absence of CL and for the number of oocytes which resumed meiosis.

Neither rat nor ovine prolactin significantly influenced the number of oocytes undergoing preovulatory maturation in ovaries without luteal tissue (control $9{\cdot}5 \pm 2{\cdot}1$, treated $10{\cdot}0 \pm 1{\cdot}8$). There was, however, a significant inverse correlation between the concentration of prolactin in the culture medium and the production of progesterone by the ovaries without CL (Table 1). The inhibitory effect of prolactin on the production of progesterone was not related to the age of the animal or to the source of prolactin. There was a significant positive correlation between the concentration of prolactin in the culture medium and the production of progesterone by the ovaries which contained CL, regardless of the age of the animal and the source of prolactin. Ovaries from 28-day-old mice which were exposed to LH + FSH produced significantly more progesterone than did controls ($P < 0{\cdot}01$, Student's *t* test), but the linear correlation between increasing concentrations of prolactin and progesterone production persisted ($r = 0{\cdot}56$, $P < 0{\cdot}01$, by linear regression analysis).

These studies confirm and extend the earlier in-vitro studies of McNatty *et al.* (1975) for human granulosa cells, and suggest that prolactin may have a direct role in regulating ovarian steroidogenesis in the mouse. High concentrations of prolactin appear to enhance steroidogenesis by the CL but inhibit that by the developing follicles. This divergent action may be important in regulating follicular development during periods of stress, during pregnancy, or in lactational anoestrus when there are elevated concentrations of prolactin in plasma (see review by Horrobin, 1974).

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Table 1. Effect of prolactin on production/24 hr of progesterone by mouse ovaries *in vitro*

Treatment		25-day-old ovaries		28-day-old ovaries	
Type of hormone added	Dose (ng/ml)	With CL	Without CL	With CL	Without CL
Control	—	54·0 ± 6·3	68·7 ± 16·5	49·2 ± 17·7	56·7 ± 10·9
Rat prolactin	50	45·0 ± 5·6	69·3 ± 21·2	—	22·6 ± 7·6
	100	73·6 ± 10·6	52·8 ± 17·8	—	15·9 ± 3·1
	500	87·0 ± 15·2	37·0 ± 9·9	—	7·6 ± 2·7
	1000	85·6 ± 10·0	23·0 ± 6·8	—	5·7 ± 1·0
Ovine prolactin	50	48·4 ± 4·2	65·3 ± 11·3	—	27·0 ± 6·5
	100	65·6 ± 8·3	53·0 ± 22·3	74·7 ± 11·0	28·9 ± 7·6
	500	77·7 ± 9·1	34·2 ± 11·8	106·4 ± 22·0	16·7 ± 3·0
	1000	87·9 ± 10·6	34·5 ± 6·5	108·4 ± 24·0	8·2 ± 4·0
	5000	—	—	162·5 ± 37·4	—
Human prolactin	50	—	—	—	33·0 ± 9·5
	250	—	—	—	22·6 ± 8·8
	1000	—	—	—	13·3 ± 4·7
FSH + LH	—	—	—	108·3 ± 15·1	—
FSH + LH + ovine prolactin	100	—	—	140·3 ± 31·6	—
	500	—	—	163·0 ± 17·1	—
	1000	—	—	230·0 ± 58·0	—
	5000	—	—	350·4 ± 63·8	—

Linear regression analysis established the following significant correlations between prolactin concentration and progesterone production:

25-*day-old ovaries*: with CL; rat prolactin, $r = 0{\cdot}45$, $P < 0{\cdot}01$; ovine prolactin, $r = 0{\cdot}52$, $P < 0{\cdot}01$; without CL; rat prolactin, $r = 0{\cdot}41$, $P < 0{\cdot}01$; ovine prolactin, $r = 0{\cdot}36$, $P < 0{\cdot}05$.

28-*day-old* ovaries: with CL and ovine prolactin, $r = 0{\cdot}48$, $P < 0{\cdot}01$; without CL, ovine prolactin, $r = 0{\cdot}58$, $P < 0{\cdot}01$, rat prolactin, $r = 0{\cdot}58$, $P < 0{\cdot}01$; human prolactin, $r = 0{\cdot}43$, $P < 0{\cdot}05$. With prolactin + LH + FSH the corresponding correlation was $r = 0{\cdot}56$, $P < 0{\cdot}01$.

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