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STIMULATION OF OVARIAN AROMATASE ACTIVITY IS INDEPENDENT OF POSTNATAL EXPOSURE TO GONADOTROPHINS*

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SUMMARY

The aim of this investigation was to examine whether stimulation of ovarian aromatase activity is affected by gonadotrophic deprivation from birth. Aromatase activity was estimated by incubating ovarian homogenates with 4-androstene 3,17-dione as substrate, followed by ether extraction and estradiol-17β determination by radioimmunoassay, In mice injected daily from birth for 10 days with antiserum neutralizing endogenous gonadotrophin followed by three days stimulation with gonadotrophins, ovarian estradiol-17β production (0.90 ± 0.02 ng/mg ovarian protein) was not significantly different from that in control littermates treated for 10 days with saline followed by 3 days with gonadotrophins. Thus ovarian competence to respond to hormonal challenge by stimulating aromatase activity is acquired independently of postnatal gonadotrophic exposure.

The effects of depriving ovaries of circulating endogenous gonadotrophins have been studied by means of daily administration of anti-gonadotrophin antiserum to mice for periods of 1–2 weeks continuously from birth [1, 2]. It was shown that despite impairment in ovarian granulosa and theca cell morphogenesis in the absence of postnatal gonodotrophic exposure, biochemical parameters such as cyclic AMP and lactic acid production remain responsive to gonadotrophic simulation [1, 2]. A rise in cyclic AMP formation should not be interpreted as proof of functional competence, however, since a number of steps are necessary for translating an increase in cyclic AMP formation into the appropriate physiological response. It was therefore of interest to test for an essential ovarian function under the same experimental conditions: aromatase activity, a key step in estrogen biosynthesis.

The *in vivo* treatment of Swiss Albino mice was identical to that described in our earlier study [2], i.e. daily subcutaneous injections (0.05 ml during the 1st week and 0.1 ml during the 2nd week) of antiserum to rat gonadotrophins (anti-rGn). Characterization of the antiserum has been described earlier [3]. Following 10 days of such treatment, a group of mice (and saline-treated control littermates) were also injected subcutaneously for 3 days with hFSH (1 IU) and hLH ((1 IU). The mice were killed at 10 and 14 days of age, and the ovaries homogenized (1:11 tissue concentration) in potassium phosphate buffer (0.075 M, pH 7.4) containing nicotinamide (0.03 M), $MgCl_2$ (1 mM) and sucrose (0.25 M). The protein concentration in the homogenates was estimated by the method of Lowry *et al.*[4]. Aromatase activity was estimated by incubating 0.1 ml ovarian homogenates with 4-androstene 3,17-dione (10 μg) as substrate (more efficient than testosterone as precursor of *in vitro* aromatization—see ref. [5] and 0.9 ml incubation medium (potassium phosphate buffer, 0.075 M, pH 7.4, and 0.03 M nicotinamide, 1 mM $MgCl_2$, 0.25 M sucrose together with a NADPH generating system: 2 mM NADP, 6 mM glucose-6-phosphate + 1 μg protein/ml glucose-6-phosphate dehydrogenase: enzyme activity which oxidizes 4 μmol/min of glucose-6-phosphate). Each ovarian homogenate was incubated in duplicates at both 0 and 60 min. (to determine *de novo* estrogen production) at 37°C with air as gas phase. Blanks consisted of substrate + incubation medium. Following incubation and ether extraction (2×10 ml ether), estradiol-17β was estimated by radioimmunoassay as previously described [6].

The results (Table 1) show that estradiol-17β was less than 0.2 ng/mg ovarian protein in 10-day-old mice injected daily from birth with anti-rGn as well as in 10- to 14-day-old saline-treated control littermates. Ovarian estradiol-17β production rose to 0.90 ± 0.02 ng/mg ovarian protein in mice treated for 10 days with anti-rGn followed by 3 days with hFSH and hLH, levels not significantly different from that in control littermates injected for 10 days with saline followed by 3 days with hFSH and hLH (0.87 ± 0.01 ng/mg ovarian protein).

Thus ovarian competence to respond to hormonal challenge by stimulating aromatase activity is acquired independently of postnatal exposure to

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Table 1. Effect of anti-gonadotrophins on mouse ovarian estradiol-17β production

In vivo treatment	Estradiol-17β† (ng/mg ovarian protein) Incubation time: 0′	60′	
10-day-old, anti-rGn treated	<0.2 (6)	<0.2 (6)	
10-day-old, saline treated	<0.2 (6)	<0.2 (6)	
14-day-old, saline treated	<0.2 (6)	<0.2 (6)	
14-day-old, saline then Gn* treated	<0.2 (6)	0.87 ± 0.01 (6)	NS‡
14-day-old, anti-rGn then Gn* treated	<0.2 (6)	0.90 ± 0.02 (6)	

* hFSH + hLH, Pergonal, Serono.
† Mean ± SEM, with number of estimations indicated within brackets.
‡ Non-significant difference ($P > 0.4$, Student's t-test).

gonadotrophins. Moreover, since according to present concepts [7] granulosa cells are the site of the aromatase reaction, it would seem that these cells retain their capacity to respond to hormonal challenge by stimulating aromatase activity despite the morphological impairment [1] induced by gonadotrophic deprivation from birth. The data are in accordance with the high rate of success with which hypogonadotrophic amenorrheic women respond to gonadotrophin treatment with estrogen formation and eventual ovulation [8].

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