Gonadotropin receptors, cAMP and testosterone in estrogenized male rats

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Summary. In neonatally estrogenized and PMSG-treated rats, binding of ^{125}I-HCG to testicular receptors correlates with levels of plasma testosterone, but not with formation of cAMP.

Neonatal treatment of male rats by single injections of estrogen produces atrophy of the testis, accesory sex organs and a decrease of spermatogenesis[1]. In our previous report, evidence was presented that the delay in sexual maturation of neonatally androgenized rats was also related to pubertal decrease of gonadotropin testicular receptors[2]. The aim of the present work has been to determine whether gonadotropin binding capacity in estrogenized rats may be recovered by long-term treatment with human chorionic gonadotropin (HCG) or pregnant mare's serum gonadotropin (PMSG), and further to correlate testicular binding of ^{125}I-HCG with formation of cyclic 3′, 5′-AMP (cAMP) and testosterone.

Material and methods. Male rats of the Wistar strain bred in our local animal room were injected on the 3rd postnatal day with 250 µg estradiol dipropionate (Agoffolin, Spofa). When 30 days old, the rats were treated either with 40 IU of HCG (Preadyn, Spofa), 30 IU of PMSG (Gestyl, Organon) or saline at 3-day intervals. At the age of 65 days (5 days after the last injection), the animals were sacrificed by decapitation. Iodination of HCG (kindly donated by Dr R. Canfield, Columbia University) and specificity of ^{125}I-HCG binding to rat testis was described previously[3]. In brief, 1500 × g testicular binding fraction (equivalent to 40 mg tissue) was incubated with 5.10^{-14} moles ^{125}I-HCG (specific activity 32 Ci g^{-1}) in 0.01 moles l^{-1} phosphate buffer (pH 7.4) with 0.14 moles sodium chloride at room temperature for 16 h. The radioactivity of washed pellets was determined by counting in gamma spectrometer. Specific binding was calculated as the difference between total binding and binding in the presence of excess unlabelled hormone. The concentration of cAMP in the testis and plasma was determined by Gilman's protein binding assay[4, 5]. The slices of rat testis were incubated for 20 min at 37 °C in Krebs-Ringer phosphate buffer with 6 mmoles l^{-1} glucose and 7.5 mmoles l^{-1} aminophylline in the presence or absence of 1 µg ml^{-1} of lutropin (LH). Testosterone in plasma was measured by the radioimmunoassay according to Stahl[6] without chromatography (antiserum kindly supplied by Dr F. Stahl, Berlin). The amount of protein in the tissue was estimated by the method of Lowry et al.[7].

Results. Specific binding of ^{125}I-HCG by testicular homogenates in neonatal estradiol-treated rats is decreased (table). A further drop of gonadotropin binding was found in rats treated with HCG as from day 30 ($p < 0.001$). In PMSG-treated rats there was an increase of gonadotropin-binding capacity above that seen in saline-injected estrogenized animals ($p < 0.01$). Concentration of cAMP in testis and plasma is decreased only in estrogenized or estrogenized and HCG-treated rats. The responsiveness of rat testis to LH in cAMP synthesis is not related to changes of gonadotropin receptors. The percentage increment of cAMP under the action of LH is elevated in estrogenized rats. The drop in plasma levels of testosterone in estrogenized rats is changed by the long-term action of PMSG ($p < 0.01$ compared to saline treated estrogenized animals).

Discussion. In agreement with previous studies[2], it has been found that neonatal administration of estradiol to male rats depresses ^{125}I-HCG binding to testicular receptors. This decline in formation of testosterone and HCG binding sites is not related to changes of plasma LH concentration, which showed little variation during postnatal life[11]. Neonatal estrogenization of rats resulted in a drop plasma FSH levels throughout the 2 month[11], suggesting that the definite concentration of FSH could be of importance for a normal development of gonadotropin receptors as well as formation of testosterone. The stimulatory effect of FSH on activation of gonadotropin receptors was demonstrated in ovarian cells[12]. Kincl et al.[8] reported that treatment of estrogenized rats with PMSG fully restored gonadal function. Our results go to show that PMSG increased gonadotropin binding and plasma concentration of testosterone in estrogenized rats, but these values are below the levels of control animals neonatally treated with oil. The marked drop of gonadotropin binding in estrogenized rats treated with HCG might be due to a decrease in availability

Specific testicular binding of ^{125}HCG, relative weight of testis and cAMP synthesis in estrogenized saline, HCG and PMSG treated 65-day-old rats

		Control	Estrogenized Saline	HCG	PMSG
^{125}I-HCG bound 10^{-15} moles/mg protein		9.1 ± 0.33 (12)	5.85 ± 0.15^c (8)	1.93 ± 0.19^c (12)	7.26 ± 0.39^a (12)
Weight of testis mg/g b.wt		9.59 ± 0.19 (6)	1.81 ± 0.18^c (12)	3.29 ± 0.20^c (8)	3.61 ± 0.17^c (9)
cAMP 10^{-12} moles/ml plasma		31.5 ± 0.67 (5)	30.6 ± 0.65 (13)	27.7 ± 0.70^b (8)	33.6 ± 1.27 (10)
cAMP 10^{-11} moles/g testis		7.1 ± 0.85 (5)	4.1 ± 0.17^a (4)	5.0 ± 0.33 (4)	5.5 ± 0.55 (4)
cAMP* 10^{-11} moles/g testis	No LH	8.76 ± 0.24 (5)	9.22 ± 1.17 (4)	9.35 ± 0.66 (4)	11.5 ± 1.24 (4)
	LH	20.5 ± 3.3 (5)	41.9 ± 8.1 (4)	24.4 ± 2.3 (4)	31.1 ± 1.9 (4)
Testosterone 10^{-12} moles/ml plasma		7.07 ± 0.81 (5)	0.46 ± 0.09^c (10)	0.77 ± 0.25^c (8)	1.79 ± 0.27^c (9)

Differences significant at *a* $p < 0.05$; *b* $p < 0.01$; *c* $p < 0.001$ from the control group with the number of observations in parentheses.
* Responsiveness of testis to LH at 20 min incubation.

of receptors, without their being saturated with exogenous gonadotropin, as shown by Sharpe[9] in the immature rat testis.

It is generally accepted that the action of gonadotropin on steroidogenesis is mediated via formation of cAMP. Testicular gonadotropin-binding in rats neonatally treated with sex hormones underwent variation which depended on the functional state of the testis[2], and represents a suitable model for studying its relationship to changes in synthesis of cAMP and testosterone. The results of the present study show that neither plasma and testis levels of cAMP, nor responsiveness of testis to LH in cAMP synthesis, correlate with testicular gonadotropin receptors and steroidogenesis. It is possible that formation of cAMP occurred in cells not involved in testosterone production, or that neonatal estrogenization may have uncoupled cAMP synthesis from formation of testosterone. Moyle et al.[14] observed that LH binding to receptors involved in cAMP synthesis may be different than those necessary for testosterone production in rat Leydig cells. Moreover, Catt and Dufau[10] showed that, with increasing gonadotropin-binding in rat testis, there is a progressive rise in cAMP synthesis, despite the fact that above 1% occupancy there is no further increase in testosterone production. In our experiments only maximal stimulatory dose of LH was used[13], and so it is difficult to explain difference between responsiveness in cAMP and gonadotropin bindig sites. However, the pattern of changes of testicular receptors in estrogenized and PMSG-treated rats is related to plasma levels of testosterone, which points to a close relationship between gonadotropin-binding capacity and steroidogenesis in the modified gonadal function.

1 F. A. Kincl, A. Folch Pi and L. H. Lasso, Endocrinology *72*, 966 (1963).
2 J. Kolena, Endocrinologie, *69*, 266 (1977).
3 J. Kolena, Endocr. exp. *10*, 113 (1976).
4 A. G. Gilman, Proc. nat. Acad. Sci. USA *67*, 305 (1970).
5 J. Kolena and C. P. Channing, Endocrinology *90*, 1543 (1972).
6 F. Stahl, Endokrinologie *65*, 288 (1975).
7 O. H. Lowry, N. J. Rosenbrough, A. L. Farr and R. J. Randall, J. biol. Chem. *193*, 265 (1951).
8 F. A. Kincl, M. Maqueo and A. Folch Pi, Acta endocr. *47*, 200 (1964).
9 R. M. Sharpe, Nature *264*, 644 (1976).
10 K. J. Catt and M. L. Dufau, Nature New Biol. *244*, 219 (1973).
11 K. Brown-Grant, G. Fink, Fenella Greig and M. A. F. Murray, J. Reprod. Fert. *44*, 25 (1975).
12 C. P. Channing, Proc. Soc. exp. Biol. Med. *149*, 238 (1975).
13 J. Kolena, Archs int. Physiol. Biochim. *83*, 627 (1975).
14 W. R. Moyle, O. P. Bahl and L. März, J. biol. Chem. *250*, 9163 (1975).

Influence of temperature and gonadal steroids on the testis composition in *Rana esculenta*[1]

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Summary. Male frogs at 28 °C show an increase in the testis weight and a decrease in its protein content. At 15 °C and 4 °C the testis lipids increase. Testosterone administration nullifies the low temperature-induced changes in the testis.

Several encyclopedic accounts have been published on the influence of temperature and hormonal compounds upon the anuran testis[2–4]. The available literature indicates that adverse environmental, hormonal and nutritional factors notably affect the anuran testis and the severity and time relationship of the response of the testis differs from species to species. It is also well understood that the spermatogenic tissue responds more readily to such factors than the interstitial tissue. Very little information is, however, available regarding the relationship between the changing testis composition and testis function.

Adult male frogs were procured from the surroundings of Naples and divided into 5 batches of 10 frogs each. 3 batches were kept at 28 °C, 15 °C and 4 °C, on a 12 h light: 12 h dark schedule, for 7 days. The remaining 2 batches of frogs, kept at 15 °C (12 h dark: 12 h light), were treated respectively with 50 µg testosterone and 50 µg estradiol-17β. 3 equal fractions of the total dose of hormones, dissolved in the physiological solution, were injected into dorsal sac on alternate days. At the time of sacrifice, frogs were killed with chloroform and the b.wt and fresh testes weight were determined. 1 testis from each animals was used for the determination of total lipids (according to de La Huerga[5]) and proteins (according to Lowry et al.[6]), whereas the contralateral testis was used for obtaining dry weight.

High temperature (28 °C) stimulates testis weight ($p < 0.05$) but decreases the water and protein contents ($p < 0.001$ and $p < 0.001$; table). The testis lipids show no significant alteration at 28 °C as compared with the initial value ($p = 0.2$). High temperature influence is always accompanied by the stimulation of spermatogenesis and regression of the interstitial tissue[3]. Frogs kept at lower temperatures (i.e. 15 °C and 4 °C) do not show any change in their testis weight but the testicular lipid concentration rises significantly ($p < 0.02$ and $p < 0.01$ respectively). At 15 °C no alteration is found in the testicular protein concentration, whereas at 4 °C a significant decrease is observed ($p < 0.001$). As far as the testis function is concerned, low temperatures usually impair spermatogenesis and stimulate the interstitial tissue[3]. Administration of testosterone to frogs at 15 °C greatly stimulates the testis dry weight (i.e. a decrease in water content) as compared with frogs at 15 °C ($p < 0.001$). On the other hand, the protein content of the testis in testosterone-treated frogs greatly decreases, and both the dry weight and protein concentra-

1 We are thankful to Prof. G. Chieffi for his criticism. Work done under the C.N.R.-financed finalized project 'Biologia della Riproduzione'.

2 B. Lofts, in: Physiology of Amphibia, vol. II, p. 107. Ed. B. Lofts. Academic Press, New York & London 1974.
3 R. K. Rastogi, Boll. Zool. *43*, 151 (1976).
4 R. K. Rastogi, L. Iela, P. K. Saxena and G. Chieffi, J. exp. Zoll. *196*, 151 (1976).
5 J. De la Huerga, in: Tecnica e diagnostica di laboratorio, vol. I, p. 287. Ed. D. D. USES, Bologna 1969.
6 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, J. biol. Chem. *193*, 265 (1951).