Changes in Protein Kinase Activity in Rat Testes during Development

By ELENA A. BERNARD and GUILLERMO F. WASSERMANN

*Instituto de Biología y Medicina Experimental, Obligado 2490,
Buenos Aires, Argentina*

(Received 15 November 1976)

Protein kinase activity in rat testes remained fairly constant from day 16½ of embryonic life up to 10 days after birth. At the 21st postnatal day a nadir of activity was observed, and after an increase at 35 days of age a decrease in activity at 60 days was seen. The enzyme reached maximal specific activity in the testes of 90-day-old rats.

In previous studies (Bernard & Wassermann, 1974; Reddi *et al.*, 1971), the presence of high specific activity of protein kinase (ATP-protein phosphotransferase, EC 2.7.1.37) in rat testes was reported. Enzymic activity increased between 35 and 45 days of age, and it was suggested that this increase in activity was related to the process of metamorphosis in which spermatids become spermatozoa. Also, it is well known that testicular synthesis and metabolism of testosterone show profound variations in late embryonic and early postnatal life. Furthermore, the blood concentrations of follitropin (follicle-stimulating hormone, FSH), lutropin (luteinizing hormone, LH) and testosterone undergo important changes from birth to maturity. With the aim of correlating protein kinase activity in testes with the variation in hormone concentrations, a detailed sequential study of protein kinase activity from day 16½ of embryonic life up to 90 days of age was carried out.

A preliminary report of protein kinase activity in rat testes during development was presented at the 8th Pan-American Congress of Endocrinology, Workshop on Endocrinology of Growth and Development, Buenos Aires, Argentina, October 1974.

Materials and Methods

Animals

Testes from male Wistar rats of different ages were used. The testes were removed, and the tunica albuginea was dissected off.

Preparation of protein kinase

The gonads were homogenized with 3 vol. of neutral 4mM-EDTA. The homogenate was centrifuged at 600 g for 15 min, and the sediment discarded.

Protein kinase assay

Protein kinase activity was determined by measuring the incorporation of phosphate from [γ - 32 P]ATP

into histone (200 µg) (calf thymus, type II; Sigma Chemical Co., St. Louis, MO, U.S.A.) by the procedure of Bernard & Wassermann (1974, 1975).

Results

Cyclic AMP-dependent and -independent forms of protein kinase activity were measured, with histone as substrate. Samples of crude testes homogenate were prepared from rats ranging from 16½-day embryos to adults. The results are shown in Fig. 1. Over the age range from the last days of foetal life to the 10th postnatal day the activity remained fairly constant. After the 10th day the specific activity decreased significantly, reaching its lowest values at the 20th day of life. From 20 to 35 days of age the activity increased slightly and from 35 to 45 days a marked increase was detected. The values at 60 days of age were somewhat lower, but at 90 days the highest values were detected (375 pmol/min per mg of protein). With histone as substrate the ratio of activation with cyclic AMP remained constant from foetal life up to 35 days of age (approx. 2.40). From 40 days to adulthood a statistically significant increase in cyclic AMP-dependence was found [i.e. at 20 days of age the ratio was 2.30 ± 0.008 , and at 45 days it was 3.45 ± 0.09 ; means $\pm$ S.E.M. of determinations on five animals; the values are significantly ($P < 0.001$) different, as evaluated by Student's *t* test].

Discussion

Our results on protein kinase demonstrate marked variations in enzyme activity during pre- and post-natal maturation, the most striking changes being the decrease in activity around 20 days of age and the sharp increase between 35 and 45 days of age. This variation correlates reasonably well with the changes in testicular adenylate cyclase activity (Hollinger, 1970), especially of the Mn^{2+} -sensitive soluble form (Braun & Dods, 1975), and also with

Fig. 1. Protein kinase activity in rat testes during development

Values represent means of three samples for embryos or five samples $\pm$ S.E.M. for postnatal animals. The phases of spermatogenesis are shown. For experimental details see the Materials and Methods section. ●, Activity in the presence of cyclic AMP; ○, in the absence of cyclic AMP.

the variation observed in phosphodiesterase activity (Monn *et al.*, 1972).

It is also useful to recall that around this period of life protein kinase becomes more cyclic AMP-dependent and that this dependence continues throughout life. This increase also coincides with the appearance of lactate dehydrogenase isoenzyme X (Blackshaw & Elkington, 1970), which is present in spermatozoa.

The profile of the variations in protein kinase activity have some parallel with the changes in testosterone biosynthesis and progesterone metabolism in testes (Noumura *et al.*, 1966; Ficher & Steinberger, 1971): in late foetal and early postnatal life (up to approx. 10 days of age) testosterone synthesis is very high. Formation of this compound diminishes with age, reaching a nadir at the 20th day. On the other hand, production of testosterone only starts to increase at 40 days of age, reaching adult values at 90 days of age.

The nadir of the protein kinase activity curve also agrees with the lowest values reported for lactate dehydrogenase isoenzyme X and Mn^{2+} -dependent adenylate cyclase activities.

Protein kinase activity during maturation does not agree precisely with the changes reported in the

literature for testosterone, follitropin and lutropin concentrations in plasma (Lee *et al.*, 1975). It seems more reasonable to link the observed variations with other parameters as well. The large increase found between 35 and 45 days of age is probably related to the appearance of a population of elongated spermatids. A correlation of this type was observed by Dixon (1972) in trout testes, whose spermatids have a high activity of protamine kinases; this activity is connected with the process of replacement of nuclear proteins during spermiogenesis.

Therefore it appears that the variations in protein kinase activity reported above are more related to the general changes that occur during differentiation and maturation than to the changes of any particular parameter.

E. A. B. and G. F. W. are established investigators of the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

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