AGE-DEPENDENT DIFFERENCES IN THE BINDING OF [131] LH BY RAT TESTIS HOMOGENATES

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The specific binding of radioiodinated HCG and LH by homogenates of adult rat testes *in vitro* has been demonstrated by Catt, Dufau & Tsuruhara (1972). The binding sites were of high affinity and the uptake of HCG was greatest at 24° C, maximum uptake occurring by 24 hr of incubation under these conditions (Catt, Tsuruhara & Dufau, 1972). There have been no reports of comparable work on the binding of LH by the testes of immature rats, but Means & Vaitukaitis (1972) have demonstrated a greater uptake of [3 H]FSH *in vitro* by immature than by mature rat testes. Changes in the characteristics of the gonadal receptors for LH could be a factor in the control of sexual maturation. The binding of 131 I-labelled human LH ([131 I]hLH) by testicular homogenates from immature and mature Porton rats was therefore compared using the methods of Catt, Dufau & Tsuruhara (1972) and Catt, Tsuruhara & Dufau (1972).

The right testis from each of three to ten rats, according to the age-group being studied, were decapsulated and homogenized in Krebs-Ringer bicarbonate buffer (KRB) using eight strokes of a loosely-fitting glass homogenizer. After filtering through cotton wool, the homogenate was centrifuged at 1500 *g* for 10 min and the sediment weighed and resuspended at a concentration of 40 mg/ml KRB. Incubations with [131 I]hLH were carried out using 0.4 ml of this suspension in a final volume of 0.6 ml in glass vials under a 95/5 mixture of O₂/CO₂ at 21° C. At the end of the experiment, the incubates were twice centrifuged after the addition of cold KRB, as described by Catt, Dufau & Tsuruhara (1972). Radioactivity was measured in the precipitate and a tissue/medium ratio (T/M) was calculated.

Highly purified human LH, suitable for radioimmunoassay (Fraction IRC2 prepared by Dr A. Stockell Hartree), was labelled with 131 I by the method of Greenwood, Hunter & Glover (1963) to a specific activity or approximately 100 mCi/mg. The weights of [131 I]hLH quoted are only approximate: for this reason and because of possible variation in the biological activity of different batches of labelled hormone, all experiments were designed as simultaneous comparisons of the uptake by tissues from immature and mature animals.

In the first series of experiments, homogenates from rats aged either 5, 22 or 130 days were incubated with [131 I]hLH (approx. 0.8 ng/ml) for 16 hr. Other tubes contained, in addition, either ovine LH (NIH.LH S17, 167 ng/ml)

or ovine FSH (NIH.FSH S8, 417 ng/ml). Uptake of [131 I]hLH was considerably higher in adults than in 22-day-old or 5-day-old rats (Text-fig. 1). The presence of FSH had little effect on uptake, but the addition of ovine LH reduced uptake in all groups. The absolute reduction was greatest in adults although in this group, T/M was not reduced to unity (Text-fig. 1). Uptake by comparable homogenates of adrenal tissue from the same animals was never significant (T/M $\sim$ 1.0) and was unaffected by the addition of ovine LH. The lower uptake of [131 I]hLH by the homogenates from immature animals suggests that these contained fewer binding sites and/or sites of lower affinity.

To obtain more information about the characteristics of these binding sites, the uptake from media containing increasing quantities of [131 I]hLH was studied. As uptake was slow, it was studied under equilibrium conditions. To ensure that these had been obtained, the length of incubation was increased

TEXT-FIG. 1. The uptake of [131 I]hLH by testis homogenates from immature and mature rats. Each block represents the mean of three readings, vertical lines representing $\pm$ 1 S.E. Hormones added during incubation are shown at the base of the appropriate columns.

to 21 hr, a time at which previous experiments had consistently shown equilibration. Otherwise, the conditions were the same as in the previous experiment except that 0.1% bovine serum albumin (Fraction V) and streptomycin and penicillin (100 units/ml of each) were added to the medium. In material from 5-day-old and 22-day-old rats, uptake of [131 I]hLH increased linearly with increasing concentration of labelled hormone in the medium (Text-fig. 2), but a much greater uptake, with evidence of saturation, was observed in homogenates from adult rats. Linear increase in binding with no indication of saturation could be due to non-specific uptake, and, to test this possibility, the experiment was repeated with homogenates from 22-day-old and from adult rats, but an excess (1 μ g/ml) of unlabelled ovine LH was added to some of the tubes. Specific uptake of the labelled LH should be largely inhibited by such an excess of unlabelled LH so that any residual uptake should be a measure of non-specific uptake. This figure was subtracted from the total uptake to provide an assessment of specific binding. Using this procedure, the results suggested

saturation in tissue from 22-day-old animals but at a much lower level of uptake (approx. 6 pg [131 I]hLH/mg tissue) than for tissue from adults in which the uptake appeared to be levelling off at about 30 pg [131 I]hLH/mg of tissue.

The same data were used to construct Scatchard plots. Those for the main experiment are shown in Text-fig. 2: those for the additional experiment (in which some tubes contained excess ovine LH) are not illustrated but showed a similar pattern. The Scatchard plots failed to show the apparently simple situation found by Catt, Tsuruhara & Dufau (1972) although the relatively steep slope of the lines describing uptake at low concentrations of [131 I]hLH in tissue from both adult and immature animals probably represents uptake by the high affinity binding sites which they identified. If this is so, then testes from immature animals presumably contain these high affinity binding sites but at a much lower concentration per unit weight of testis than in the adult.

TEXT-FIG. 2. Left: Uptake of [131 I]hLH by homogenates from rats aged (a) 150 days, (b) 22 days, (c) 5 days. Vertical lines represent ± 1 S.E. Right: The same data shown as a Scatchard plot.

Measurement of the uptake of LH by homogenates of whole testis does not distinguish between uptake by tubular and interstitial tissue. The uptake is, however, peculiar to gonadal tissue and specifically blocked by excess ovine LH. This suggests that, even under the unphysiological conditions used, the LH is bound to physiological receptor sites. Again, equilibrium at 21 hr must reflect factors other than simply binding, e.g. breakdown of labelled hormone. Incubation at the low temperature of 21° C was selected because it has been shown by Catt, Tsuruhara & Dufau (1972) to encourage maximum uptake of HCG in their system. It may be that a low temperature simply decreases the rate of hormone breakdown which would otherwise mask uptake.

Even with these qualifications, it seems that sexual maturation in male rats is accompanied by an increase in testicular binding sites for LH. These findings fit well with the increase in Leydig cells between 20 and 50 days of age in rats

shown by Knorr, Vanha-Perttula & Lipsett (1970), as these cells presumably contain the LH receptors. Our results also suggest that the low level of testosterone secretion in immature male rats (Knorr *et al.*, 1970) is not necessarily due to a low level of circulating LH, but that the secretion of androgen may also be limited by the availability of receptor sites for LH.

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