Short communication

Age-Dependent Changes in Rat Testicular LH/hCG Receptors in Relation to the Membrane Fluidity

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Changes in membrane-associated testicular LH/hCG receptors in prepubertal, adult and aged rats have been extensively studied (Kolena 1976; Ketelslegers et al. 1978; Geisthövel et al. 1981). The cell membrane is a dynamic matrix which responds to various physiological conditions by changing its physical state. Receptors are a heterogenous population of molecules which interact with membrane components, and their movement within the membrane may be modified by changes in lipid fluidity of the membrane. In a recent publication, we have reported that the accessibility of LH/hCG recepotors depends on the lipid fluidity of testicular membranes (Kolena et al. 1983). Since sexual maturation and senescence of rats could be brought in connection with age-dependent alterations of the membrane structure, the relationship between the testicular receptors and the membrane fluidity was investigated.

Specific binding of saturating concentrations of [^{125}I] hCG (specific activity 1.20 TBq g^{-1}) to crude testicular membranes, and plasma levels of testosterone were determinated as previously reported (Kolena 1976; Šeböková and Kolena 1978). The animals were killed by decapitation, and decapsulated testis were homogenized in cold 0.01 mol.l^{-1} phosphate buffer (pH 7.4) with 0.14 mol.l^{-1} sodium chloride (PBS), followed by centrifugation at $20{,}000 \times g$ for 15 min. The crude membrane fraction was dispersed with PBS to a concentration of 200 mg testicular tissue ml^{-1}. The order parameter (S) and the approximate rotational correlation time (τ_c) were calculated from EPR spectra (Gaffney 1976) using two different nitroxide derivatives of stearic acid, 5-doxylstearic acid I(12,3) and 16-doxylstearic acid I(1,14). Briefly, 10 μg of spin label were mixed with 1 ml of the crude membrane fraction and centrifuged at $1000 \times g$ for 5 min. ESR spectra were recorded using an ERS 230 spectrometer. Typical instrumental setting were: 5 mW microvave power, modulation amplitude 2×10^{-4} T (in order parameter measurements) and 1×10^{-4} T (in correlation time measurements). The order

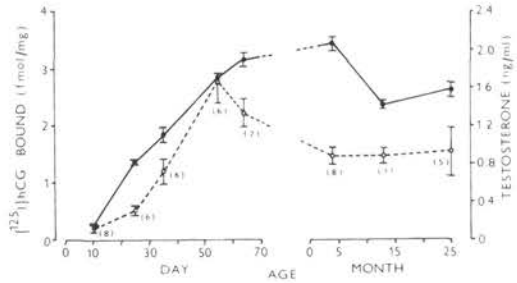

Fig. 1. Age related changes in [^{125}I] hCG specific binding (●——●) to a crude testicular membrane preparation, and plasma testosterone levels (O- - - -O). The bars indicate standard error of 8 estimations.

parameter was calculated from the outer ($2A_{max}$) and inner ($2A_{min}$) splittings after $A_\perp$ and polarity correction, respectively. The order parameter, S, is a measure of the distribution of molecular orientation of the spin label relative to the normal (to the membrane surface). The lower the S, the less regularly the acyl chains extend towards the membrane interior. S is a normalized quantity ($0.1 \leqslant S \leqslant 1$).

Because of the complexity of the membrane system, a decrease in S is interpreted as an increase in the fluidity close to the polar part of the membrane.

A progressive rise in the specific binding of [^{125}I] hCG to the crude testicular membrane fraction was observed from day 10 through 64 (Fig. 1). The maximum binding in this period corresponded with both, the maximum testis size and the highest number of the Leydig cells in the testis (Pahnke et al. 1975). No change in specific binding was evident after 4 months; a significant fall was noted there after (following 13 months) ($p < 0.001$); it remained constant through month 25. Since FSH stimulates the formation of new LH/hCG receptors, the low level of FSH in old rats (Bruni et al. 1977) could be the cause of the decrease of [^{125}I] hCG specific binding in the aged rat testis. The increase in LH/hCG receptors during the early postnatal period precedes the pubertal rise in testosterone plasma levels. The concentration of circulating testosterone reached a peak between days 55—64, and decreased in month 4 ($p < 0.05$) to attain a plateau in aged rats. The decrease in the plasma testosterone concentration is in agreement with the findings of Tsitouras et al. (1979) who observed a decrease in testicular activity between 90 days and 22 month of life, but Geisthövel et al. (1981) found such a decrease in andvanced senescence of rats (39 months). The pubertal rise of [^{125}I] hCG binding to the testicular membranes is not closely correlated with the plasma LH concentration; the latter rises relatively slowly in the course the development (Ketelslegers et al. 1978), and it is rather related to an increase in the number of the Leydig cells, and, at least partly, to the functional state of testicular membranes. The order parameter of I (12,3)-labeled crude testicular membranes was measured at 25 °C and 32 °C as

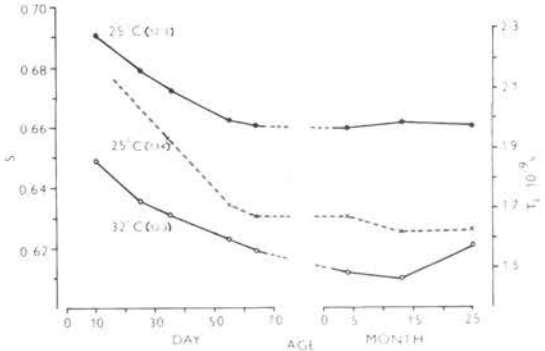

Fig. 2. Developmental changes in order parameters S (————), and rotational correlation time τ_c (– – – – –) of testicular membranes labelled with 5-doxyl-, or 16-doxylstearic acid, I (12,3) or I (1,14). The values are mean of 2 estimations.

a function of age (Fig. 2). Increasing temperature resulted in a decreased order parameter for the 5-doxyl label. A progressive decline of the order parameter occured from day 10 until the adult age at both temperatures. The order parameter increased in the aged rats (25 month) at 32 °C. Lipid dynamics in the hydrophobic core of testicular membranes was measured using I (1,14). As the membrane fluidity was much higher at the carbon-16 position, rotational correlation time was calculated. The rotational correlation time is proportional to the microviscosity of the hydrocarbon core of the membrane. During ontogenesis, the relative changes of membrane fluidity found with I (1,14) probe were very similar to those found with I (12,3) probe. Our results indicate that in rats fluidity of the testicular membrane increases with age until maturity. Significant positive correlations between the membrane fluidity measured with 5-doxyl label at 25 °C ($r = 0.964$, $p < 0.001$) and 32 °C ($r = 0.898$, $p < 0.01$) respectively and LH/hCG receptors during the entire life span was found. Increasing membrane fluidity should result either in an exposure of additional functional receptors, which are inaccessible to the hormone in more rigide membranes, or in an increased lateral diffusion rate, which would rise the changes of the hormone-receptor interaction.

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