Short communication

Effect of Gonadotropin on Rat Testicular Membrane Lipid Fluidity

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The gonadotropin receptors are located within the matrix of the plasma membrane. There is a growing body of evidence indicating that the fluidity of the lipid environment in which a receptor is embedded can change the properties of the latter. In previous studies we have shown that the LH/hCG receptor in rat undergoes variations which depend on the fluidity of the testicular membranes (Kolena and Ondriaš 1984; Kolena et al. 1983). There are several reports showing that in vitro additions of insulin and growth hormone can elicit changes in the plasma membrane fluidity in adipocytes (Sauerheber et al. 1980; Luly and Shinitzky 1979). As yet, nothing is known whether an alteration of the physical properties of the plasma membranes is involved in the action of human chorionic gonadotropin (hCG). The present study brings evidence that hCG binding to rat testicular membranes is not accompanied by changes in the lipid microviscosity.

Adult male Wistar rats were killed by decapitation. Homogenates of decapsulated testes in ice-cold 0.05 $mol\,l^{-1}$ phosphate (pH 7.4) with 0.15 $mol\,l^{-1}$ sodium chloride (PBS) were filtered through 6 layers of surgical gauze, centrifuged at $100 \times g$ for 15 min and the supernatant was further centrifuged at $20{,}000 \times g$ for 30 min (Kolena 1976). The pellet was resuspended in the same buffer (200 mg of tissue per ml), and 0.1 ml aliquots of testicular membrane fractions were incubated with 0.1 ml PBS + 1 $mg\,ml^{-1}$ bovine serum albumin with or without 100-fold excess of unlabeled hCG and 0.1 ml [^{125}I] hCG (1—1.5 ng, specific activity about 2.3 $TBq\,g^{-1}$), at room temperature for 16 h. After the incubation, pellets were washed twice with 2 ml of cold PBS (Kolena and Šeböková 1983). Results were expressed as specific binding per mg protein. Protein was determinated by the standard procedure (Lowry et al. 1951). A fluorescence polarization probe, 1,6-diphenyl-1,3,5-hexatriene (DPH) was used to monitor fluidity properties of the lipid regions of the testicular membranes. Fluorescence polarization was measured with an Aminco Bowman SPF spectrofluorometer (Shinitzky and Inbar 1976). Crude testicular membranes (100 μg protein) were incubated with 2 ml of DPH (2 $\mu mol\,l^{-1}$) in PBS buffer for 1 h at 24 °C. Fluorescence polarization was computed according to the equation:

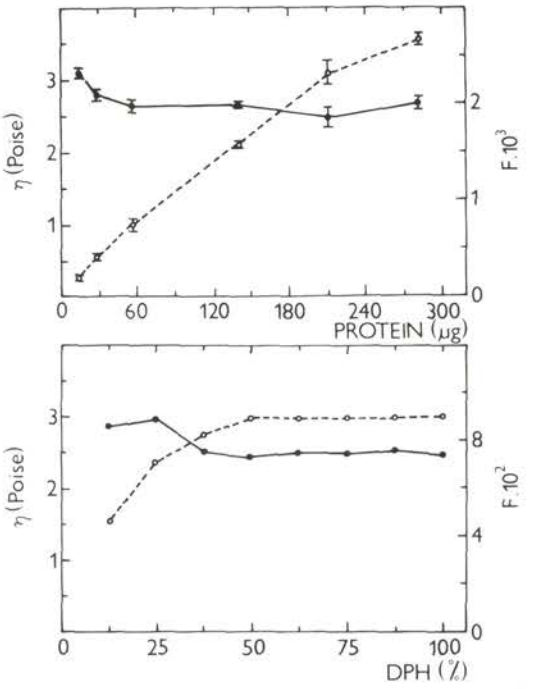

Fig. 1. The depencence of microviscosity (η, ●——●) and fluorescence intensity (F, ○- - - -○) on DPH concentration (*lower panel*) and protein (*upper panel*) in crude testicular membranes. Two μmoll^{-1} of DPH are taken for 100 %. Mean ±S.E. of 3 observations.

$$P = \frac{I_{vv} - I_{vh}(I_{hv}/I_{hh})}{I_{vv} - I_{vh}(I_{hv}/I_{hh})};$$

where I_{vv} and I_{vh} are the fluorescence intensities detected through a polarizer oriented parallelly and perpendicularly to the direction of vertically polarized light, respectively. I_{hv}/I_{hh} is the fluorescence intensity ratio at the excitation being polarized horizontally and the emission being observed through the analyser oriented perpendicularly and parallelly, respectively. The lipid microviscosity was estimated by empirical relation $2P/(0.46 - P)$ (Heron et al. 1980).

The fluorescence intensity of DPH labeled membranes increased proportionally with increasing proportion of the membrane, as might be expected (Fig. 1), but microviscosity was constant over a wide range of protein concentrations (from 30 μg protein per sample). It should be noted that fluorescence polarization is an intrisic parameter and consequently it virtually is independent of the fluorescence

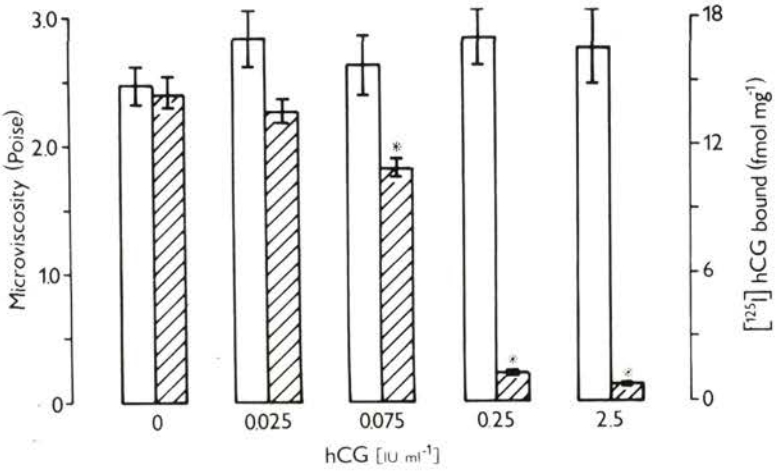

Fig. 2. Effect of increasing amounts of hCG on microviscosity (open columns) and specific binding of [^{125}I] hCG (hatched columns) to rat testicular membrane preparations. Asterisks indicate statistically significant differences ($P<0.01$). Values are mean ±S.E. of 4 determinations. These results were confirmed by two independent experiments.

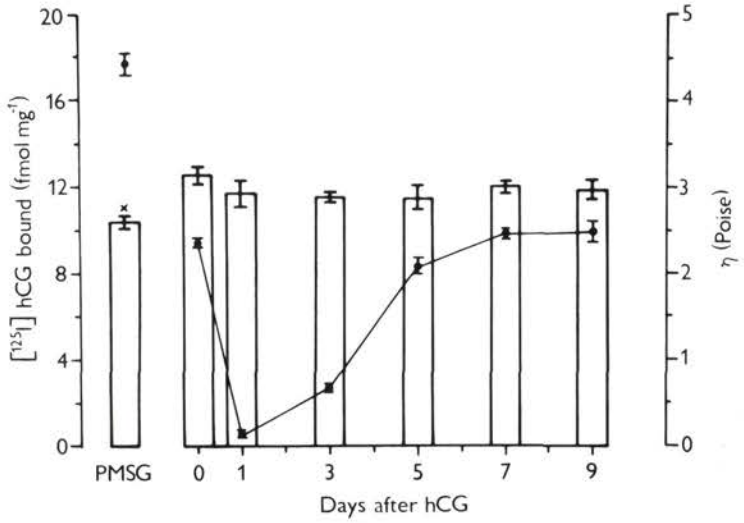

Fig. 3. Effect of treatment of rats with 60 IU of hCG (Praedyn, Spofa) or 40 IU of PMSG (Organon, OSS) on microviscosity (columns) and [^{125}I] hCG specific binding (circles) to testicular membranes. Each value is the mean ±S.E. of 6 observations in 4 rats.

intensity. A dilution of DPH up to 37 % of the initial concentration did not influence fluorescence polarization either.

The data presented in Fig. 2 show that additions of various amounts of non-labeled hCG resulted in occupation of [^{125}I] hCG binding sites without any change in the microviscosity of testicular membrane lipids. Testicular membranes previously exposed to hCG did not show altered fluorescence emmision spectra (data not shown). A similar effect of hCG was also observed with desensitizated testicular tissue. To induce a loss of LH/hCG receptors, 25-days-old rats were injected with 60 IU of hCG. A single hCG injection was followed by a 95 % reduction of LH/hCG receptor on day 1 (Fig. 3). Testicular binding was recovered on days 3 and 5, and the restoration of receptors to the control values was complete on day 7. The reduction of LH/hCG receptor one day after the injection of the hormone is not a result of endogenous occupancy by the hormone; rather, it is due to the disappearance of receptors from the Leydig cell surfaces (Kolena and Šeböková 1983). It is believed that the receptor is internalized with its ligand via small endocytotic vesicles (Ascoli and Puett 1977) and the restoration is probably dependent on the synthesis of new receptors. However, this dissappearance of LH/hCG receptors was not associated with alterations of the microviscosity of membranes lipids. On the other hand a repeated 5-fold treatment (every second day) of rats with 40 IU of pregnant mare serum gonadotropin (PMSG) appreciably increased the formation of LH/hCG receptors with a simultaneous decrease of membrane lipids microviscosity ($P<0.01$, Fig. 3). The stimulatory effect of PMSG on the receptors is in agreement with previous findings (Kolena and Šeböková 1983); however, as far as fluidity of membrane lipids concerned, is our findings differed from the fluorescence studies by Strulovici et al. (1981) who reported that continued exposure of cultured granulosa cells to FSH increased membrane microviscosity.

Observations concerning the action of other hormones on the fluidity of membrane lipids are contradictory. Luly and Shinitzky (1979) reported that addition of insulin to a suspension of DPH labeled rat liver plasma membranes resulted in increased lipid microviscosity. Sauerheber et al. (1980) observed no change in lipid fluidity when insulin or growth hormone were directly added to rat adipocyte ghosts. The absence of any effects of hCG on fluorescence polarization of testicular membranes suggests that alterations of the membrane structure which occur during the interaction of the hormone with the receptor do not lead to changes in lipid fluidity. DPH, being hydrophobic, is an almost ideal probe for measuring lipid fluidity. It however occupies a region near the center of the bilayer (Engel and Prendergast 1981). This fluorescence label of membrane lipids may thus be incorporated in an environment other than that of the LH/hCG receptor. The membrane is heterogenous and the lipids in the receptor domain may be more,

or less fluid than this average, so that a modulation of lipid fluidity by hCG in this region cannot be ruled out.

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