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REGULATION OF AN 8-kDa PEPTIDE INVOLVED IN TESTOSTERONE PRODUCTION BY LUTEINIZING HORMONE IN RAT LEYDIG CELLS

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Summary: Results of Western blot analysis carried out with an interstitial cell extract from male guinea pig and ovarian extract from immature female rats administered equine chorionic gonadotropin (eCG) provide supportive evidence to our earlier suggestion that an 8-kDa peptide is involved in acquisition of steroidogenic capacity by the rat Leydig cells. It was found that though the signal was observed in other tissues such as liver, kidney and lung which do not produce gonadal hormones, the peptide was modulated only by lutenizing hormone (LH) in the rat Leydig cells.

Key words: Leydig cell, Steroidogenesis, Peptide and steroidogenesis.

Introduction:

Regulation of testosterone production by Leydig cells is under acute and trophic control of LH (1). Several studies have been carried out to elucidate the role of LH in regulation of enzymes involved in the synthesis of testosterone (2) and also the involvement of proteins in LH-stimulated steroidogenesis (3). In a recent study (4) we demonstrated an inverse relationship between testosterone production and the presence of a 8-kDa peptide in rat Leydig cells. It was also observed that LH/human chorionic gonadotropin (hCG) administration to immature rats resulted in a decrease in the quantity of the peptide as assessed by Western-blot analysis of the Leydig cell extract. Furthermore, decrease in the endogenous LH in the adult rat by administration of testosterone resulted in an increase in the quantity of the peptide in the rat Leydig cells. Antiserum raised to the peptide exhibited a cross reaction with the Leydig cell extracts from mouse, hamster and guinea pig (4). In the present paper further studies using guinea pig, which produces maximum testosterone and the gonadotropin-primed female rat as models which provide additional support for the conclusion on the involvement of the 8-kDa peptide in the regulation of testosterone production are reported.

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Materials and methods

Immature (21-day-old) and adult (90-day-old) male and female rats of Wistar strain were used. Immature guinea pig (4 to 5-week-old) was obtained from the Central Animal Facility. The production and characterization of antiserum to the 8-kDa peptide isolated from purified rat Leydig cell has been described earlier (4).

Effect of administration of hCG/eCG on the 8-kDa peptide.

Among several rodents the guinea-pig Leydig cell has the largest area occupied by smooth endoplasmic reticulum (SER) and it is known that the ability to produce testosterone is directly proportional to the area occupied by SER in the Leydig cell (5). Guinea-pig was thus considered as to be an appropriate model to verify the hypothesis that the 8-kDa peptide is involved in the regulation of testosterone production and that it is modulated by LH. Accordingly, immature guinea pigs were injected with hCG (10 IU)/day for 10 days and interstitial cells were prepared as described earlier (4) for Western blot analysis. It is well established that administration of eCG to immature rat results in stimulation of follicular maturation and steroidogenesis. Immature rats (23-day-old) were treated with 20 IU of eCG or saline and animals were sacrificed 48 h. later; ovaries were homogenized in 0.25 μM sucrose and the supernatant obtained following centrifugation of the homogenate at 10000 "times" g for 10 min was used for Western-blot analysis (6) using antiserum to the 8-kDa peptide.

Earlier studies had indicated that there is a negative correlation between the absence of the peptide and steroidogenic potential of the tissue. In order to obtain additional support for the above conclusion, other tissues, such as heart, kidney, brain, liver and lung, which do not normally produce gonadol steroids, were checked for the presence of the 8-kDa peptide and its modulation if any by hCG/LH.

In view of the fact that an intense signal corresponding to 8-kDa peptide was detected by immunoblot in the lung extract from immature rat it was of interest to examine whether the lung peptide was also modulated by LH as in the case of Leydig cell peptide. Immature rats were injected subcutaneously with saline or hCG (1.3 IU/day) for 10 d. and sacrificed 24 h. after the last injection and the lung extract was used for immunoblotting studies.

In addition, experiments were designed to check whether the peptide was part of the LH receptor because it was specifically modulated only in the Leydig cell. Immunoblot analysis was carried out with solubilized ovine LH receptor (7) to check whether the 8-kDa peptide formed a part of the LH receptor.

It is well known that besides LH, other hormones follide stimulating hormone (FSH) and prolactins (PRL) also play an important role in the regulation of steroidogenesis. Thus the presence of specific receptor for PRL on rat Leydig cell and the permissive role of PRL in stimulation of testosterone production has been well documented (8). In contrast, no specific receptor for FSH has been demonstrated on rat Leydig cells although FSH is known to influence steroidogenesis indirectly through its action on Sertoli cells (3). In view of this, experiments were designed to examine the effects of administration of FSH or PRL on the 8-kDa peptide in immature rats. Animals received either oFSH (100 ng) or hPRL (1 μg) for a period of 10 d. and the Leydig cell homogenates were subjected to immuoblot analysis as described earlier.

Results:

Western blot analysis of the cytosolic extract of the Leydig cells from rats treated with hPRL or FSH revealed no significant change in the intensity of the signal compared to the saline treated group indicating the specificity of the effects of LH (data not provided).

Western-blot analysis of immature guinea-pig testicular interstitial cells revealed an intense signal comparable to the signal obtained with immature male rat while no signal was seen with adult pig interstitial cells and this was found to be not due to insufficient protein load as no signal was seen even when 1.2 mg of protein was analysed (Fig.1).

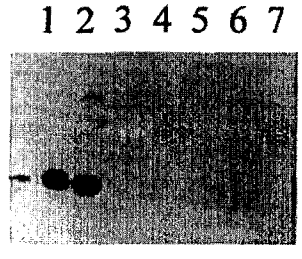

Fig.1: Western blot analysis of immature guinea pig interstitial cells extract with antiserum to the 8-kDa peptide. The antiserum was used at a dilution of 1:1000. 150 μg protein was loaded in lane 1 and 2. Lane 1: Leydig-cell extract from immature male rat; Lane 2: Interstitial cell extract from immature guinea pig; Lanes 3-7: Increasing concentrations of protein (150, 300, 600, 900 and 1200 μg) from adult guinea pig interstitial-cell extract. Antigen-antibody complex was visualized by employing iodinated protein-A.

Using hCG- and eCG-treated immature guinea pig and immature female rat, respectively, additional evidence for involvement of LH in the modulation of the 8-kDa peptide was obtained. It can be seen from the results presented in Fig.2 that while an intense signal can be seen in the cells from untreated immature guinea pig, interstitial cell extract the signal was absent in the cells from hCG-treated animal. It is known that eCG induces follicular maturation as well as steroidogenesis in immature female rat and the result presented in Fig.2, reveal that while the signal for the 8-kDa peptide can be detected in untreated animals, there was a significant decrease in eCG treated group thus once again

establishing an inverse relationship between the presence of this peptide and acquisition of steroidogenic capacity. An additional line of evidence for this conclusion is the observation that this peptide could not be detected in other steroidogenically active tissues like placenta, adrenal and corpus luteum (data not provided). This observation raised the additional possibility that this peptide could be present in other non-steroidogenic tissues. Interestingly, this was found to be true as demonstrated by results presented in Fig.3, which revealed the presence of this peptide in a variety of tissues including lung. However, the fact that the peptide was modulated only by LH in the Leydig cells was established by the observation that the intensity of the signal for the 8-kDa peptide in the lung did not change while the signal from interstitial cells from the same animal was significantly decreased (Fig. 4). This observation suggested the possibility that the 8-kDa peptide may be part of the LH receptor as it is modulated only in steroidogenic tissues, such as corpus luteum, placenta, Leydig cells which all have LH receptors. However, Western-blot analysis using antiserum to the LH receptor revealed no cross reaction induction that the peptide is not part of LH receptors (data not provided).

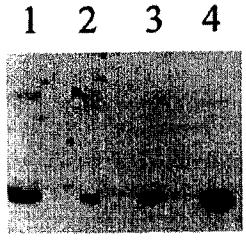

Fig.2: Effect of administration of eCG/hCG on the intensity of the 8-kDa peptide as assessed by Western-blot analysis (100 μg of protein was loaded in each lane). Lane 1: Ovarian extract from untreated immature female rat; Lane 2: Ovarian extract from immature female rat administered 20 IU eCG and 48 h. later ovarian extract was used for Western blot analysis; Lane 3: Interstitial cell extract from immature guinea pig administered hCG (10 IU) for 10 d; Lane 4: Interstitial cell extract from untreated immature guinea pig.

Discussion

Earlier studies based on electrophoretic analysis of the cytosolic proteins of immature and adult rat Leydig cells had established the possible involvement of a 8-kDa peptide in the acquisition of the testosterone-producing ability of Leydig cells (4). We were

able to provide additional support for the above conclusion. We employed two model systems, first, the guinea pig which is known to produce maximum testosterone (5). The fact that we were not able to demonstrate the presence of the peptide in spite of 1.2 mg protein being analysed in the Western-blot showed that the failure to detect the signal is not due to inadequacy of the protein loaded. In the second model the 8-kDa peptide could be detected in the immature rat, but administration of eCG which is known to stimulate steroidogenesis, resulted in its, suggesting an inverse relationship between the two.

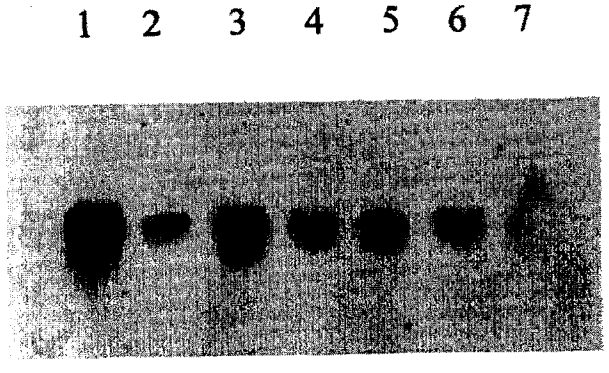

Fig.3: Western-blot analysis of extracts from different tissues of immature-rat. 100 μg of protein from each tissue was analysed Lane 1: Immature-rat interstitial-cell extract; Lanes 2-7: Muscle, lung, liver, kidney, brain and adrenal.

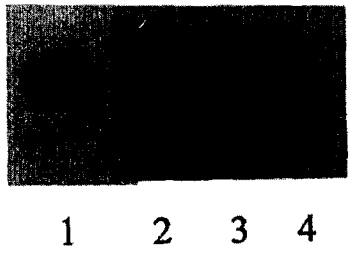

Fig.4: Lack of regulation of the 8-kDa peptide in immature male rat by hCG. Effect of administration of hCG to immature male rat the 8-kDa peptide in the lung. 100 μg of protein was analysed. Lane 1: Lung extract from immature male rat administered hCG; Lane 2: Leydig-cell extract from untreated immature male rat; Lane 3: Leydig-cell extract from immature male rat administered hCG; Lane 4: First trimester human placental extract.

It was noted that while the peptide could be detected easily in Leydig cells isolated from immature rat, mouse, hamster and guinea pig which produce relatively less testosterone, compared to adult animals it could not be detected in all the steroidogenically active tissues such as human placenta, ovine, corpus luteum and adrenal. The observation that the peptide is present only in steroidogenically active endocrime tissues suggested the possibility that, it may be present in other tissues that are steroidogenically not active. To examine this possibility, extracts from other sources such as heart, lung, liver and kidney were analysed by Western blot and it was observed that the peptide could be detected in all the above extracts except in the adrenal which is steroidogenically very active.

The presence of the peptide in non-steroidogenic tissues especially at high concentrations in the lung raised the possibility that the peptide could be a nonspecific factor with no relevant physiological function. However, the observation that the peptide was modulated by LH only in the Leydig cell but not in the lung suggested that the peptide was not a nonspecific factor. The reason for the presence of the peptide in all other tissues especially at high concentration in the lung is not known at present.

With the inherent problem of absence of a suitable biological assay for the peptide which was decreased by LH we had to resort to Western blotting for all the conclusions on the decrease of the peptide following hCG administration. However, it should be pointed out that in all the studies, an equal quantity of protein was analysed and the intensity was compared with reference to the control in that particular analysis. Considering the fact that the peptide was present in both steroidogenic and non-steroidogenic tissues it is not unreasonable to assume that the peptide may be part of an overall mechanism in deciding whether cholesterol should be channeled into steroid hormone production or not.

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