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Potentiation by Epidermal Growth Factor of the *in vitro* HCG Stimulation of Testicular Steroidogenesis in Hamsters

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Epidermal Growth Factor (EGF) has been reported to stimulate or inhibit steroidogenesis in murid Leydig cells depending on the experimental conditions used. In the present study, testicular fragments from an adult cricetid rodent, the Syrian hamster, were incubated with various doses of mouse EGF (0-2.0 μg/ml media), in the presence or absence of HCG (0-12.5 mIU/ml media). Although EGF alone did not affect *in vitro* testicular steroidogenesis, it significantly potentiated the HCG-induced elevation of the accumulation of testosterone and 17-hydroxyprogesterone in the media. In contrast, the effect of HCG on media progesterone concentration was not affected by EGF. Since in the Syrian hamster intracellular calcium loading functions as a gonadotropic stimulus, the present results could be a consequence of the EGF-induced increase in cellular calcium levels.

Key words: Testes, EGF, HCG, Syrian hamsters, Testosterone, Progesterone, Hydroxyprogesterone

Epidermal Growth Factor (EGF = Urogastrone. McKusick 131530) is a 53 amino acid peptide (MW = 6041), first described by Cohen in 1962, that has a wide variety of functions at the level of the cell membrane, cytosol and nucleus, including intracellular calcium loading (3, 8-9, 16, 23). Human EGF is coded for by a gene located on chromosome #4 (4q25), whose product is a very large transmembrane protein of 1207 amino acids that is

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the precursor of EGF and contains 7 EGF-like sequences (9, 12, 15, 29). Cellular responses to EGF can occur within a few minutes, or as late as several days after exposure to EGF (13). Binding of EGF to its membrane receptor induces a variety of tyrosine as well as threonine and serine phosphorylations which can stimulate a variety of cellular functions (12). The actions of EGF can be cyclic nucleotides-dependent or independent, and the presence of a nuclear EGF receptor has been postulated (12). The membrane EGF receptor (EGF-R McKusick 131530) is a glycoprotein (MW = 170,000) with a single 1186 amino acid polypeptidic chain. It has a large extracellular domain, a single membrane-spanning region, and a tyrosine kinase activity-containing cytoplasmic domain. The concentration of EGF-R varies depending on the cell type (20,000-200,000/cell), but it is absent from hemopoietic cells (7, 9, 16, 21, 29). In the human testis, EGF-R are present in high concentrationsin the Leydig cells and interstitial fibroblasts, but are not observed in Sertoli cells (26). The human EGF-R is coded for by a gene on chromosome #7 (7p13-p12), and this locus includes the *ERB-B* oncogene (15-16, 21). EGF stimulates both the degradation of internalized EGF-R, and their *de novo* synthesis (11).

The present study was undertaken to determine if EGF has a role in testicular steroidogenesis of cricetid rodents, and if this role is a stimulatory or inhibitory one, since, as it will be discussed later, there appears to be some controversy about the actions of EGF at the gonadal level.

Materials and Methods

Adult (> 3 month old) male Syrian hamsters (*Mesocricetus auratus*) were obtained from Charles River-Lakeview, and housed in polycarbonate cages with free access to food and water. The animal room had controlled temperature (22 ± 2 °C), and long-photoperiod illumination (16 h light/24 h).

Hamsters were sacrificed by decapitation. Testes were removed, decapsulated, weighed and cut each into six fragments of similar size. Testes fragments were incubated with mouse EGF (mEGF; 0, 0.02, 0.2 or 2.0 μg/ml media) and/or HCG (0, 3.125 or 12.5 mIU/ml media), for 4 h at 32 ± 1 °C, in Krebs-Ringer bicarbonate buffer with glucose (1 mg/ml), and in a 95 % O_2: 5 % CO_2 atmosphere, using a Dubnoff metabolic incubator (10, 28, 32).

The measurement of testosterone was performed using liquid-phase radioimmunoassay (RIA), and that of progesterone by solid-phase RIA, as described previously (4, 32). The determinations of 17-hydroxyprogesterone and estradiol were also done by solid-phase RIA. Since these kits use standard curves based on human serum, parallelism between the standard curves and curves made from pooled aliquots of Syrian hamster testes incubation media was determined.

Data from the RIA were obtained using the RIAPLOT and RIADOSE programs (3). Data were evaluated by two-way analysis of variance using the SPSS-X software on an IBM mainframe, and tested for normality of distribution by the Kolmogorov-Smirnov test, for homogeneity of variance by Barlett's test, and log- or square-root transformed as required (17-19, 24).

Results

As expected, incubation of Syrian hamster testes fragments with HCG caused an increase in the media concentrations of progesterone, 17-hydroxyprogesterone and testosterone (fig. 1). Estradiol was not detectable in testes incubation media from Syrian hamsters. Addition of mEGF alone had no effect on the media levels of these steroids (fig. 1). However, when the highest dose of

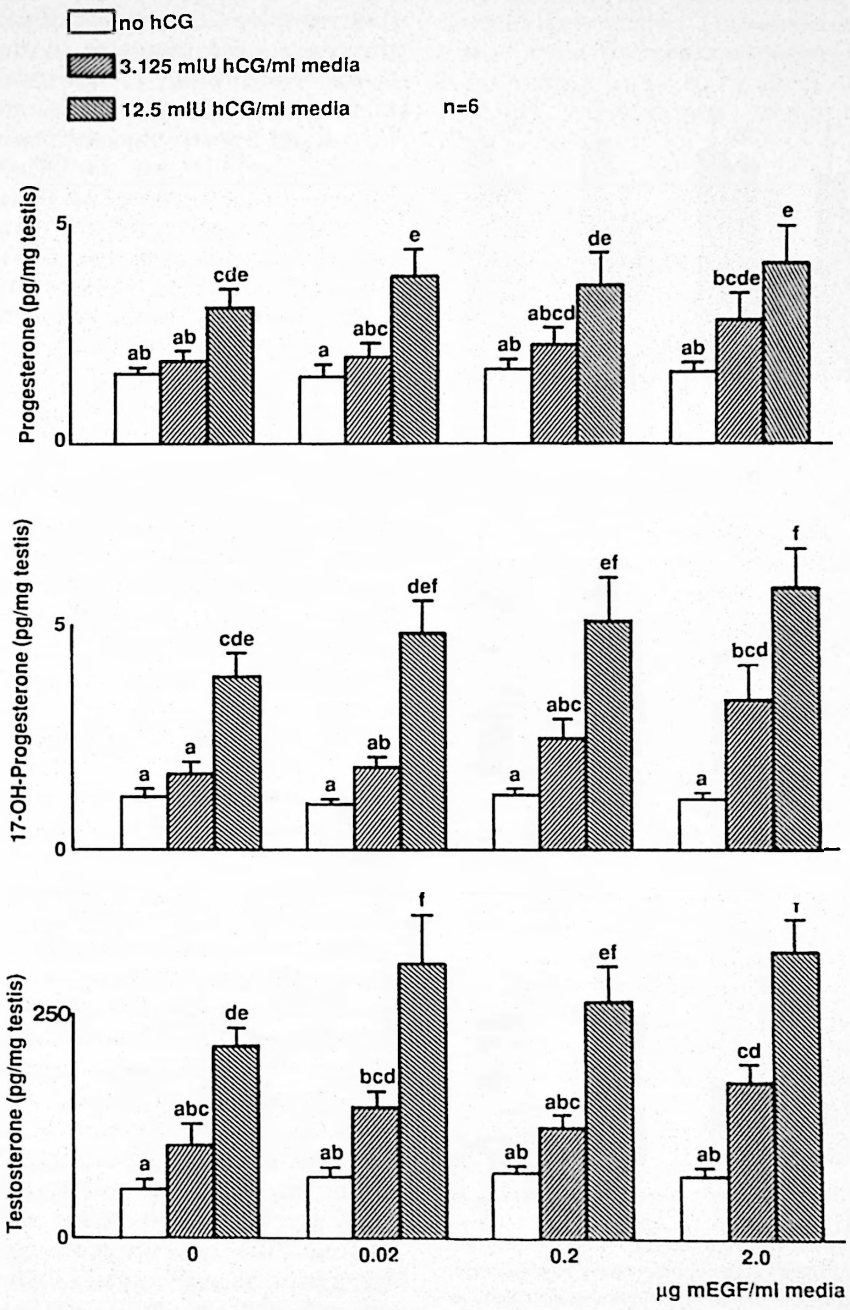

Fig. 1. *Testes incubation media progesterone, 17-hydroxyprogesterone, and testosterone levels as a function of the doses of mEGF and HCG.*
Within each panel, points with the same letter in superscript are not significantly different ($P > 0.05$) from each other (Student-Newman-Keuls procedure of the multiple range test).

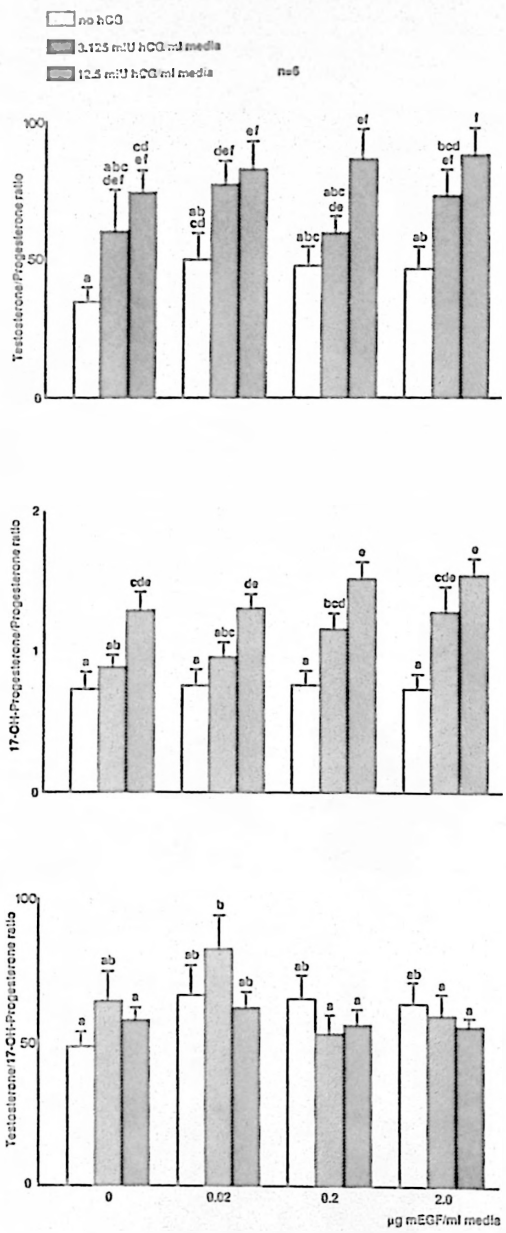

Fig. 2. *Testes incubation media testosterone/progesterone, 17-hydroxyprogesterone/progesterone, and testosterone/17-hydroxyprogesterone ratios as a function of the doses of mEGF and HCG.* Within each panel, points with the same letter in superscript are not significantly different ($P > 0.05$) from each other (Student-Newman-Keuls procedure of the multiple range test).

mEGF and HCG (at either of the two doses employed) were added together into the media, the increases in the levels of testosterone and 17-hydroxyprogesterone, but not progesterone, were higher than those measured in the media containing HCG alone (fig. 1). Whereas HCG increased the efficiency of the conversion of media progesterone into testosterone, mEGF did not alter this effect (fig. 2). This effect of HCG was due to an increase in the efficiency of the conversion of 17-hydroxyprogesterone into testosterone, and not to an effect on the efficiency of the conversion of progesterone into 17-hydroxyprogesterone (fig. 2). However, in the presence of 3.125 mIU HCG/ml media, the ratio of progesterone/17-hydroxyprogesterone was significantly increased by the highest dose of EGF used. Conversion efficiencies were determined by the respective product hormone to precursor hormone ratios.

Discussion

Previous studies on the effects of EGF on testicular steroidogenesis have used isolated normal or abnormal Leydig cells from murid rodents (mice and rats). In mouse MA-10 Leydig tumour cells, EGF stimulates or inhibits steroidogenesis depending on the duration of the exposure to EGF (5-6, 14, 20). When rat Leydig cells were studied, those that had been in culture for a prolonged period of time would respond to EGF by a reduction in testosterone synthesis (31). In contrast, steroidogenesis was stimulated by EGF in rat Leydig cells that had been freshly isolated or been in culture for a short period of time (30). Furthermore, recent studies using porcine and human Leydig cells demonstrate that in these two species EGF stimulates basal testosterone production, and potentiates the effects of HCG on this parameter (25, 27). The present results indicate that in the Syrian hamster, a cricetid

rodent, EGF does not appear to be able to stimulate steroidogenesis. However, EGF does potentiate the steroidogenic effect of HCG in this species. The differences from the data obtained in other species could be due to several reasons. Since Leydig cell function is indeed very different among species, this could account for the discrepancies (2, 4). Another reason could be that in the present study testicular fragments were used, thus conserving paracrine relationships, whereas in the previous studies isolated cells were used. Therefore, the integrity of one or more paracrine regulatory mechanisms might be required for EGF to be able to stimulate testicular steroidogenesis. Finally, since EGF is produced by Sertoli cells (26), it is possible that basal steroid levels measured in the present study already reflect the effects of EGF, whereas in the case of isolated cells, the only EGF they would be exposed to would be the one added to the media. However, the data from non-rodent Leydig cells appear to indicate that the most important factor might be species-specificity.

There are two mechanisms by which it has been proposed that EGF acts on Leydig cell steroidogenesis. EGF is capable of affecting steroidogenesis by modulating LH/HCG-dependent adenylate cyclase, and also by cyclic nucleotide-independent mechanisms such as intracellular calcium overload (5, 12, 14). There is no doubt that calcium is a potent gonadotropic agent in Syrian hamsters and rats, both *in vivo* and *in vitro*. Calcium is capable of stimulating not only steroidogenesis, but also the metabolism of other Leydig cell components such as LH receptors and inhibin (1, 22). In hamsters, calcium is more than able to compensate for low circulating levels of LH (1). Therefore, it could be proposed that the action of EGF on Syrian hamster Leydig cells is mainly through an increase in intracellular calcium. This does not imply that in murid rodents EGF could not act mainly by modulating the gonadotropin-dependent adenylate cyclase. This could be specially true if the discrepancies among the different studies prove to be species-dependent versus experimental conditions-dependent.

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Resumen

El factor de crecimiento epidérmico (EGF) estimula o inhibe, dependiendo de las condiciones experimentales, la esteroidogénesis en las células de Leydig de roedores múridos. En el trabajo presente, fragmentos testiculares de hamsters sirios adultos, se incuban con diversas dosis de EGF (0-2,0 µg/ml), con o sin HCG (0-12,5 mlU/ml). Aunque el EGF sólo no estimula la esteroidogénesis testicular, en combinación con la HCG, el EGF aumenta la producción de testosterona y de 17-hydroxiprogesterona en respuesta a la incubación con HCG. El aumento de los niveles de progesterona en el medio de incubación inducido por HCG, no se afecta por la presencia de EGF. Dado que en el hamster sirio, el aumento en la concentración intracelular de calcio actúa como estímulo gonadotrópico, los presentes resultados podrían explicarse como una consecuencia del incremento de calcio celular inducido por EGF.

Palabras Clave: Testículos, EGF, HCG, Hamsters sirios, Testosterona, Progesterona, Hidroxiprogesterona.

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