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GONADOTROPIN RECEPTOR REGULATION IN HYPOPHYSECTOMIZED RAT LEYDIG CELLS

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SUMMARY. The number of gonadotropin receptors decrease in the Leydig cells following hypophysectomy (hypox). The receptor number is reduced to 52, 48, 11, 10 and 12 % of the control 8 days following hypophysectomy in 40, 50, 60, 70 and 80 days old rats respectively. hCG injection (0.6 or 30 µg) produces a decrease in the receptor number in 58 days old hypox rat. Receptors remain almost undetectable between 24 to 72 hours following hCG injection. Desensitization to hCG is observed between 12 and 48 hours and full responsiveness to hCG is obtained at 60 hours following hCG injection (0.6 µg).

The results demonstrate that LH is not a necessary condition for the presence of gonadotropin receptors in the Leydig cells and that hCG induces the "down regulation" of the receptors and the desensitized state as well in the hypox as in the intact animal. They also indicate that a variation in the number of gonadotropin receptors is probably not the major biochemical alteration responsible for steroidogenic refractoriness in Leydig cells.

INTRODUCTION. The number of gonadotropin receptors has been shown to be controlled by LH and hCG in the intact animal. A single injection of LH or hCG induces a decrease in the number of gonadotropin receptors in the Leydig cells of the testis (1-4) as well as in the corpus luteum of the ovary (5, 6). The phenomenon of "down regulation" of the receptor by its own hormone is maintained for several days. Other hormones in addition to gonadotropins for example FSH and prolactin, participate in the regulation of the number of gonadotropin receptors in Leydig cells (7, 8) and in the ovary (9).

It is well known that following hypophysectomy in the adult, basal and hCG induced testosterone secretion are reduced (10). This decrease may be explained by a deficit in the enzymes involved in steroidogenesis (11, 12) and possibly by a decrease significant (13, 14) or moderate (15, 16) in the number of gonadotropin receptors. However, replacement therapy with LH, FSH or prolactin fails to maintain or restore the normal binding capacity (13, 16).

In order to elucidate the mechanism of gonadotropin receptor regulation and cell responsiveness to gonadotropin we have studied the effect of hypophysectomy and hCG injection on gonadotropin receptors number and testosterone production in the Leydig cells.

MATERIALS AND METHODS. Hypophysectomized male Sprague-Dawley rats, aged 40 to 80 days were used in all experiments. The adequacy of the hypophysectomy was confirmed by examination of the sella turcica and measurement of testosterone

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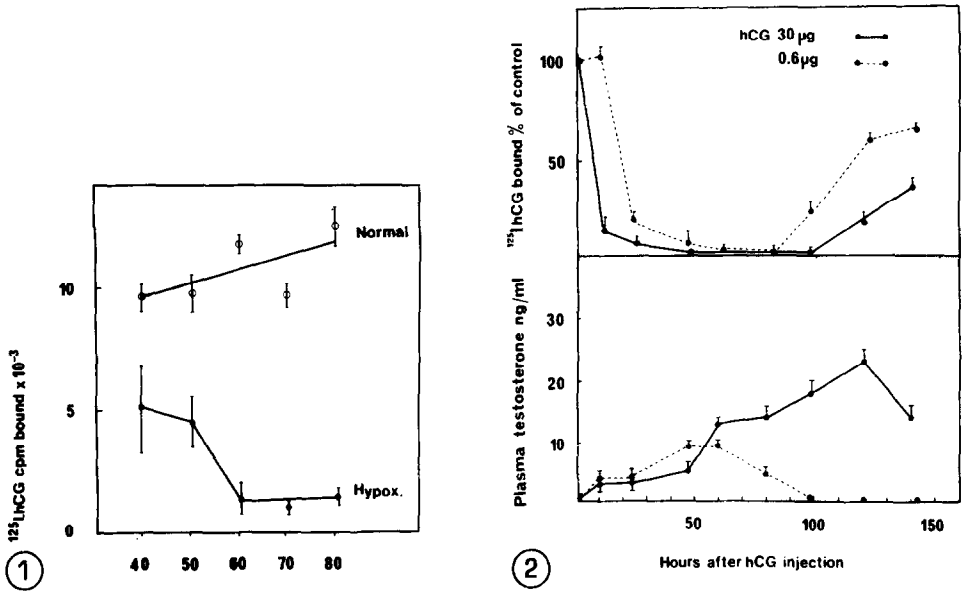

Figure 1 : Specific binding of ^{125}I-hCG (cpm/100 µg protein) of particulate preparation prepared from whole testis at different ages (O—O). The same measurement was achieved 8 days following hypophysectomy at day 40 to 80 (●—●).

Figure 2 : Variation of ^{125}I-hCG binding sites in particulate fractions prepared from isolated Leydig cells (upper panel) and of plasma testosterone (lower panel) following one injection of hCG (0.6 or 30 µg hCG). Experiment were made 8 days following hypophysectomy at day 50.

and corticosterone plasma levels. Leydig cell preparation were obtained after collagenase dissociation of the testis as previously described (17). Particulate fractions (18) containing the plasma membranes were prepared from the isolated Leydig cell preparation. Iodination of hCG (15,000 IU/mg, from Dr. Bosch, the Netherlands, and CR 115, gift from the NIH) was achieved by the lactoperoxidase method (18). Measurement of the binding capacity of the membrane preparation was achieved as previously described (3). Plasma testosterone was extracted with ethyl ether. The extract was purified on a microcelite column and testosterone measured using a specific radioimmunoassay (19). (1,2,6,7-^{3}H)-testosterone (SA 90 Ci/mM) and carrier free Na-^{125}I were from Amersham, England. Pregnyl(R) (hCG) used for the treatment of the animals was from Organon.

RESULTS AND DISCUSSION : Effect of the time of hypophysectomy on the variation of the receptors in the Leydig cells.

Figure 1 indicates that intact rats acquire nearly adult number of gonadotropin receptors as early as 40 days of age. This fact is in agreement with

those of others (20). The decrease in the number of receptors observed 8 days following hypophysectomy (Fig. 1) is dependent on the time of the operation. When achieved at day 40 or 50 the number of receptors remains close to 50 % of the control value. When achieved at day 60 or more the loss of receptors is close to 90 %. In the case of animals hypophysectomized at 40, 50, 60, 70, 80 days of age plasma testosterone was respectively 0.04, 0.09, 0.04, 0.03, 0.05 ng/ml of plasma on the eighth day following the operation.

A sharp shift in the regulation of the cell receptors clearly occurs between days 50 and 60. It is of interest that this period corresponds to the attainment of sexual maturity. Whatever the explanation for this shift, the results indicate that the presence of LH and other pituitary hormones is not necessary before day 50 for the maintenance of a significant number of gonadotropin receptors.

- Variation of plasma testosterone and hCG receptors following one injection of hCG. After hypophysectomy plasma testosterone was, in all cases, below 0.1 ng/ml. The injection of hCG (0.6 or 30 µg) to rats hypophysectomized at 50 days of age produces a moderate increase in testosterone for 48 hours (Figure 2, lower panel). The plasma levels remain below 10 ng/ml and are clearly inferior to the levels observed in intact animals (Fig. 3, left panel). However, a delayed stimulation occurs after 48 hours (Fig. 2, lower panel). At 120 hours following the injection of 30 µg of hCG plasma testosterone reaches 25 ng/ml. In the case of the low dose (0.6 µg) this peak is not observed. The delayed peak of plasma testosterone observed in hypox rats after the injection of 30 µg of hCG is similar to that described in intact animals (21, 22). This peak is probably due to hCG still present in plasma since it has been already shown (3) that the biological half life of hCG is close to 16.5 hours. Following the injection of 0.6 µg of hCG concentrations are however too low to induce any delayed steroidogenesis.

The number of gonadotropin receptors in the Leydig cells of rats hypophysectomized at day 50 represents 50 % of the control level (Fig. 1). As shown in Fig. 2 (upper panel) this number is sharply reduced following the injection of 30 µg of hCG and reduced, but with some delay, following the injection of 0.6 µg of hCG. Within 48 hours, in both cases, the number of receptor sites is very low and remains so until 72 hours. Reappearance of the receptor occurs around 96 hours and is faster in the case of the low dose of hCG.

As observed in the intact animal (1-3) a phenomenon of "down regulation" of the receptors by its own hormone is present in the hypox animal. Since the "down regulation" of the receptor is simultaneous to a period of desensitization to hCG stimulation (21, 22) we have investigated whether such a desensitization is also present in hypox animals.

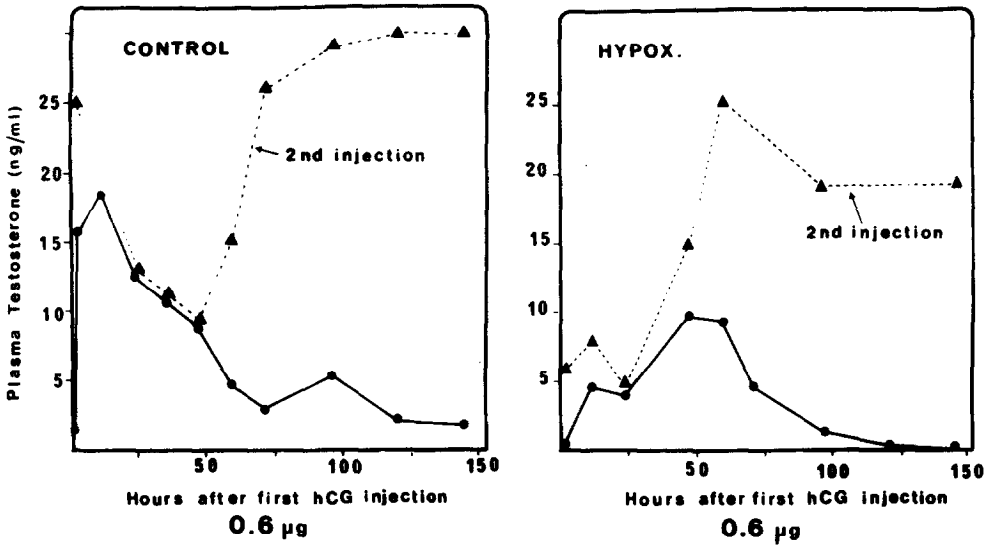

Figure 3 : Effect of one injection (solid lines) and 2 injections (dotted lines) of hCG on plasma testosterone in intact (left panel) and in hypox rats (5 days). The second injection (30 µg of hCG) was administered (3 rats for each determination) 2 hours before the animals were killed by decapitation for blood collection.

- In vivo desensitization to hCG in hypophysectomized animals. In the intact animal the desensitization period lasts for 48 hours after the injection of 0.6 µg of hCG. As shown in Fig. 3 (left panel) the plasma testosterone level observed following one dose of hCG (solid line) is not significantly increased by a second injection (dotted line) between 24 and 48 hours. After 48 hours the normal response reappears. In the hypox animal (Fig. 3, right panel) the basal level of plasma testosterone is low (0.09 ng/ml) but one injection of hCG (solid line) multiplies the testosterone level by more than 20. The cells are then responsive to hCG although at a very low level. Complete refractoriness to a second hCG injection (dotted lines) is observed at 24 hours and partial refractoriness at 12 and 48 hours. At 60 hours the response is almost similar to normal and remains so until 144 hours.

It seems that the Leydig cells which have been deprived of gonadotropins for 8 days maintain a certain number of hCG receptors and the capacity to be stimulated, although at a very low level, by hCG. The phenomenon of "down regulation" of the receptors and of refractoriness to hCG is similar to that observed in the intact animal (21, 22). The reappearance of sensitivity to hCG is observed after 2 days following the injection as in the intact animal. At that time the cells have recovered from the desensitized state and the positive

effect of hCG on the enzymes necessary for steroidogenesis can be expressed (10-12). Full testosterone response to hCG stimulation may then be obtained in the hypophysectomized animal. Since this full response can be observed (50 to 72 hours following the injection of 0.6 µg of hCG) in the absence of a detectable number of gonadotropin receptors one must conclude that, in the hypox as well as in the intact animal, the number of the gonadotropin receptors is probably not the major biochemical alteration responsible for steroidogenic refractoriness in Leydig cells.

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