HEPARIN FACILITATES THE INDUCTION OF LH RECEPTORS BY FSH IN GRANULOSA CELLS CULTURED IN SERUM-ENRICHED MEDIUM

A. NIMROD and H. R. LINDNER
Department of Hormone Research, The Weizmann Institute of Science, Rehovot, Israel

Received 28 July 1980

1. Introduction

The development of the ovarian follicle in mammals is regulated by the sequential secretion of FSH and LH [1]. While the granulosa cells (GC) carry receptors for FSH during early stages of follicular development [2], LH receptors appear on GC only during the final stages of follicular growth [3,4]. FSH induces the appearance of LH-receptors in GC of rat preantral follicles in vivo [5] and in organ culture [6]. By contrast, GC from similar preantral follicles maintained as monolayers in serum-enriched medium did not respond to addition of FSH with increased LH binding, although the cells responded to FSH with increased progestin production [6]. These findings led to the suggestion that participation of other cellular components of the ovary, or their products, are required for the induction of LH receptors by FSH in GC. However, it has been shown that functional LH receptors can be induced by FSH in isolated GC provided they are cultured in a chemically-defined medium devoid of serum [7]. Since in vivo the follicle is exposed to most serum proteins, we examined whether the follicular fluid contains a factor that counteracts the inhibitory action of serum on the induction of the LH-receptor. We show that heparin, which is a constituent of the mucopolysaccharides that are abundant in follicular fluid (total 2 mg/ml; [8,9]), supports the induction by FSH of LH receptors in GC cultured in the presence of serum.

2. Materials and methods

Granulosa cells from hypophysectomized immature rats that had been treated with diethylstilboestrol (Hx-DES) were cultured as in [10]. The medium was either devoid of serum or supplemented with 10% serum derived from hypophysectomized rats. In some cultures, serum was substituted by lipoprotein-deficient serum, prepared from the same batch by differential centrifugation [11]. Rat FSH (0.1 μg rFSH-I-3/ml, kindly supplied by the National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, MD), heparin (grade 1, Sigma, St Louis), chondroitin sulfate A or C (Miles Labs, Kankakee, IL) and dextran sulfate (M_r 500 000; Sigma), were added where indicated. Cells ($5–8 \times 10^5$) were cultured in Falcon 60×15 mm tissue culture dishes with 3 ml of medium. At the end of the culture period, the medium was collected and the cells incubated for 30 min in 3 ml 0.14 M NaCl, 10 mM potassium phosphate, 10 mM EDTA and 0.1% bovine serum albumin (pH 7.4). The cells were then harvested with a rubber scraper, the cell suspension was combined with the original tissue-culture medium and sedimented by spinning at $500 \times g$ for 10 min. The cells were washed once, and a portion was used for assay of ^{125}I-hCG binding as in [4,6]. The remainder was used for measurement of hCG-stimulable cyclic AMP accumulation as in [4].

3. Results and discussion

In agreement with the results in [7], GC from Hx-DES rats cultured for 48 h in the presence of rFSH in serum-free medium acquired a significant number of hCG binding sites (a 6.6-fold increase of ^{125}I-hCG binding over control; $p < 0.001$; fig.1A), while cells maintained in serum-enriched medium did not respond to FSH, and hCG-binding even slightly declined. When heparin (0.2 or 1 mg/ml) was included in the serum-enriched medium, the cells responded to

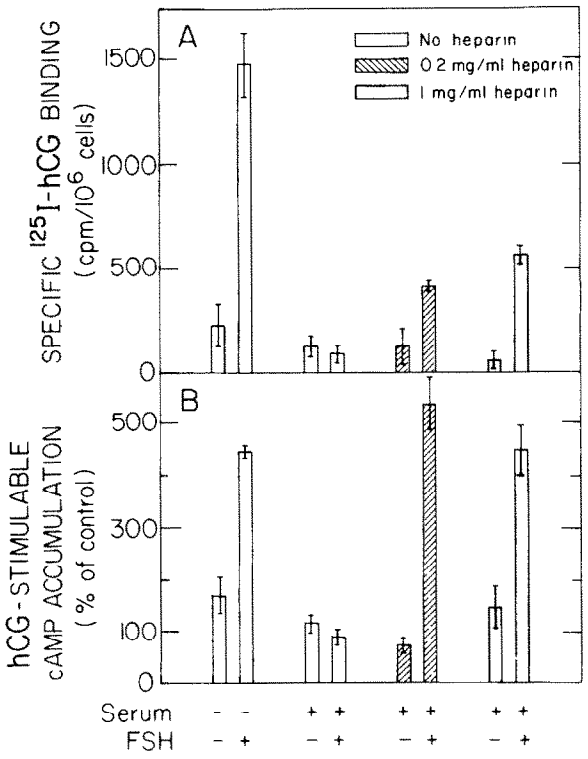

Fig.1 The effects of serum and of heparin on the induction by FSH of (A) ^{125}I-hCG binding and (B) hCG-stimulable cAMP accumulation in cultured granulosa cells from Hx-DES rats. Cells (2.5 × 10^6/3 ml) were cultured for 48 h in medium supplemented, where indicated, with 10% serum from hypophysectomized rats, 0.1 μg/ml rFSH or heparin (0.2 or 1 mg/ml). For each treatment combination, cells from three dishes were combined and washed, and their capacity for ^{125}I-hCG binding (n = 4) and for hCG-stimulable cAMP accumulation (n = 3) were assayed. Mean values ± SEM (vertical brackets) are shown.

FSH with increased ^{125}I-hCG binding ($p < 0.05$ and $p < 0.01$, respectively). The extent of the binding was only 27–38% of that obtained by FSH in serum-free medium. This increase of hCG binding was associated with the appearance of hCG-stimulable cAMP accumulation (fig.1B), indicating that the newly acquired receptors are biologically active. Moreover, the extent of hCG-stimulable cAMP accumulation by cells treated concomitantly with FSH, serum and heparin was similar to that obtained with cells treated with FSH in the absence of serum (fig.1B), in spite of the greater number of binding sites present on the latter cells (fig.1A). This finding suggests that the amount of LH receptors induced by FSH in the presence of serum and heparin may be adequate for the expression of a maximal biological response, consistent with the concept of 'spare receptors' [12]. Addition of heparin on its own to serum-enriched medium (fig.1A) or heparin together with FSH to serum-free medium (not shown) had no effect on the binding capacity of the cells for hCG after 48 h culture. Another mucopolysaccharide, chondroitin sulfate C, also facilitated induction of the LH-receptor, but its potency was only ~50% that of heparin (not shown). Its isomer, chondroitin sulfate A, and dextran sulfate, a synthetic sulfated polysaccharide, were devoid of activity.

It has been shown [13] that heparin and related substances can interfere with hCG binding to ovarian cell membranes and inhibit LH-stimulable adenylate

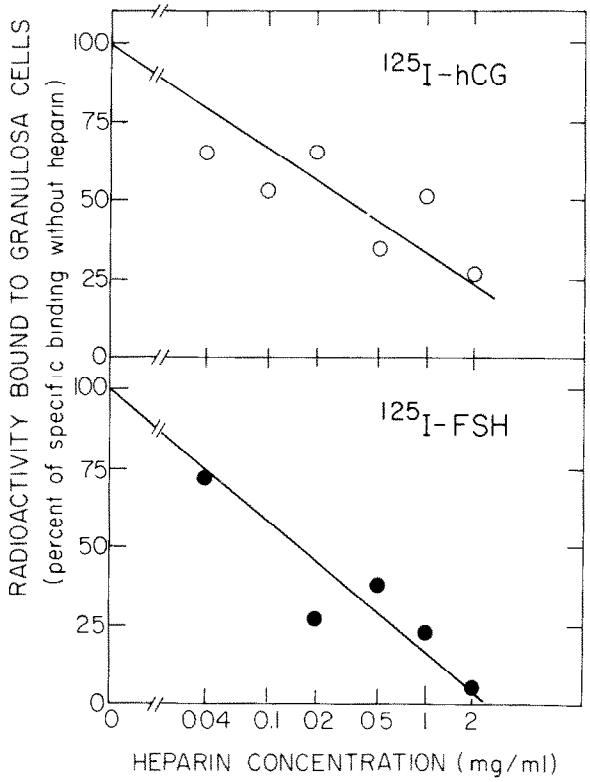

Fig.2. Inhibition by heparin of the binding of ^{125}I-hCG and ^{125}I-FSH to granulosa cells. Cells (4 × 10^5/0.1 ml), collected from immature rats 48 h after injection of 15 IU pregnant mare serum gonadotrophin, were incubated with labelled hormone (50 000 cpm/sample) in the presence of increasing amounts of heparin. The amount of labelled hormone bound in the presence of a 100-fold excess of the homologous unlabelled hormone was deducted to get the specific binding capacity. The specific binding in the absence of heparin was taken as 100%. Each point represents the mean of 3 determinations.

cyclase activity. We examined the effect of heparin on ^{125}I-hCG and ^{125}I-FSH binding to intact cells from pregnant mare serum gonadotrophin-treated rats. The presence of heparin (0.04–2 mg/ml) in the binding buffer inhibited the binding of both gonadotrophins in a dose-dependent manner (fig.2).

The nature of the inhibitory substance in serum and of the factor in follicular fluid abolishing its activity remains to be elucidated. It is possible that even after hypophysectomy the serum contains traces of gonadotrophins, and that these induce internalization of newly formed hCG binding sites during culture [14]. Such an effect may be suppressed by heparin, since this agent inhibits the binding of hCG (and, by implication, of LH) to its receptor (fig.2). This would leave, however, the question why FSH retains its ability to induce LH-receptors in the presence of heparin, in spite of the inhibitory action of heparin on FSH binding (fig.2). Perhaps minute amounts of FSH suffice for the expression of its receptor-inducing activity, whereas a higher degree of receptor occupancy may be required for desensitization [15]. A more plausible explanation of the results is that heparin may bind to a putative serum inhibitory component and thus prevents it from interfering with the induction of the LH-receptor. Heparin has been shown to bind to various serum components, such as antithrombin [16], complement factors [17], enzymes [18] and lipoproteins [19]. An involvement of serum lipoproteins in the inhibition of LH-receptor induction appeared to be an intriguing possibility, since these compounds are believed to play a role in the cellular transport of cholesterol [20] and thus may influence membrane structure and fluidity. However, addition of lipoprotein-deficient serum to the culture medium inhibited the induction by FSH of the LH-receptors to the same extent as whole serum (not shown).

The results reported here establish an interaction between FSH, serum components and heparin in the induction of the granulosa cell LH receptor in vitro. Further work is needed to clarify whether the native sulfated glycosaminoglycans that occur in follicular fluid have a significant physiological role in the ontogeny of the LH receptor.

Acknowledgements

This work was supported by grants of the Ford Foundation, the Population Council and the Rockefeller Foundation to H.R.L.

References

[1] Richards, J. S. and Midgley, A. R. jr (1976) Biol. Reprod. 14, 82–94.
[2] Presl, J., Pospisil, J., Figarova, V. and Krabec, Z. (1974) Endocrinol. Exp. 8, 2–9.
[3] Channing, C. P. and Tsafriri, A. (1977) Metabolism 26, 413–468.
[4] Nimrod, A., Bedrak, E. and Lamprecht, S. A. (1977) Biochem. Biophys. Res. Commun. 78, 977–984.
[5] Zeleznik, A. J., Midgley, A. R. jr and Reicher, L. E. jr (1974) Endocrinology 95, 818–825.
[6] Nimrod, A., Tsafriri, A. and Lindner, H. R. (1977) Nature 267, 632–633.
[7] Erickson, G. F., Wang, C. and Hsueh, A. J. W. (1979) Nature 279, 336–338.
[8] Zacharie, F. and Jensen, C. E. (1958) Acta Endocrinol. 27, 343–355.
[9] Gebauer, H., Lindner, H. R. and Amsterdam, A. (1978) Biol. Reprod. 18, 350–358.
[10] Nimrod, A. and Lindner, H. R. (1976) Mol. Cell. Endocr. 5, 315–320.
[11] Havel, R. J., Eder, H. A. and Bragdon, J. H. (1955) J. Clin. Invest. 34, 1345–1353.
[12] Catt, K. J. and Dufau, M. L. (1977) Ann. Rev. Physiol. 39, 529–557.
[13] Salomon, Y., Amir, Y., Azulai, R. and Amsterdam, A. (1978) Biochim. Biophys. Acta 544, 262–272.
[14] Amsterdam, A., Nimrod, A., Lamprecht, S. A., Burstein, Y. and Lindner, H. R. (1979) Am. J. Physiol. 236, 129–138.
[15] Lamprecht, S. A., Zor, U., Salomon, Y., Koch, Y., Ahren, K. and Lindner, H. R. (1977) J. Cyclic Nucl. Res. 3, 69–83.
[16] Miller-Andersson, M., Borg, H. and Andersson, L.-O. (1974) Thromb. Res. 5, 439–452.
[17] Von-Zeipel, G., Hanson, H.-S. and Von Stedingk, L.-V. (1977) Acta Pathol. Microbiol. Scand. C85, 123–130.
[18] Boberg, J., Augustin, J. and Baginsky, M. L. (1977) J. Lipid Res. 18, 544–547.
[19] Iverius, P.-H. (1972) J. Biol. Chem. 247, 2607–2613.
[20] Brown, M. S. and Goldstein, J. L. (1979) Proc. Natl. Acad. Sci. USA 76, 3330–3337.