REVISTA ESPAÑOLA DE FISIOLOGIA, 51 (4), 187-192, 1995

Effects of Somatostatin and Somatotropin on the *in vitro* Testicular Steroidogenesis in Hamster

S. S. Oh[1], R. Khardori[2], D. K. Kopplin[2] and A. G. Amador[1]

[1]Division of Research, Department of Obstetrics & Gynecology, SIU School of Medicine, Springfield, IL 62794-1315 (USA)

[2]Division of Endocrinology, Metabolism and Nutrition, Department of Internal Medicine, SIU School of Medicine, Springfield, IL 62794-1612 (USA)

(Received on June 9, 1995)

S. S. OH, R. KHARDORI, D. K. KOPPLIN and A. G. AMADOR. *Effects of Somatostatin and Somatotropin on the* in vitro *Testicular Steroidogenesis in Hamster.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 51 (4), 187-192, 1995.

Adult hamsters were exposed to short-photoperiod, and injected with either somatotropin (GH), somatostatin (GHRIH), or saline for eight weeks. Hamster testis fragments of similar size were incubated with or without hCG. No significant differences in the basal media testosterone and estradiol levels were observed among groups. Treatment with GH potentiated the hCG-dependent increase in media testosterone. Contrary to what was expected, treatment with GHRIH did not only not reduce the hCG-related elevation in media testosterone, but even produced a numerical increase of it. Treatment with GHRIH potentiated the hCG-dependent increase in media estradiol, whereas treatment with GH produced only a numerical increase of the response. Furthermore, the combined exposure to GHRIH and hCG appeared to cause an increase in the efficiency of testicular aromatase. Since previous data indicated that the combined deficiency of lactotropic and somatotropic actions severely impairs testicular steroidogenesis, treatment with GHRIH should have caused further steroidogenic impairment in hamsters exposed to short-photoperiod. Since this does not appear to be the case, it could be postulated that GHRIH has a direct stimulatory or at least a protective effect on testicular steroidogenesis.

Key words: Testes, Somatostatin, Somatotropin, hCG, Syrian hamster, Testosterone, Estradiol.

Correspondence to A. G. Amador, M. D., Division of Research, Dept. Obstetrics & Gynecology, SIU School of Medicine, P.O. Box 19230, Springfield, IL 62794-1315, aamador@siumed.edu (USA).

Although much is known about the effects of prolactin (PRL) on the gonads, less is known about the effects of other lactotrophic hormones on the reproductive system. Furthermore, the somatotrophic axis appears to be more complex than the lactotrophic axis, since most of prolactin effects are direct, but not so for somatotropin. The somatotrophic axis includes hypothalamic, pituitary, placental, and target organ components. Somatoliberin (GHRH; growth hormone releasing hormone) and somatostatin (GHRIH; growth hormone release inhibiting hormone) constitute the hypothalamic component. Somatotropins (GH; growth hormone) I and II, together with the GHRH (GHRH-R) and GHRIH (SST-R) receptors constitute the pituitary component. GH I and II, together with chorionic somatomammotropins (CSH: placental lactogen) I and II, constitute the placental component. The target organ component includes both circulating and cellular molecules. These are somatomedins (IGF) I and II, somatotropin (GH-R), somatomedin (IGF-R) and somatostatin (SST-R) receptors, somatotropin (GHBP) and somatomedin (IGFBP) binding proteins, somatocrinins (GHRH-like peptides or peripherally synthesized GHRH), and somatostatin. Somatotropin stimulates testicular function directly and/or through the action of IGF-I synthesized by the liver (24, 25, 30, 33). Production of a somatocrinin, of larger molecular weight than hypothalamic GHRH, has been detected in rat germ cells (10, 12, 31, 37, 40) and in mouse testis (38). Somatoliberin appears to be produced in human and rat Leydig cells, and in rat germ cells (12, 14, 31). Less is known about GHRIH, except that it is produced throughout the male reproductive tract, including the Leydig cell (30, 33).

Human GH has been shown to have potent lactogenic activity (15, 18, 19, 22). Thus, treatment with hGH is perceived by rodents as administration of both PRL and GH, and should have similar effects as those seen in hyperprolactinemia, which stimulates testicular function in Syrian hamsters (5). In contrast, exposure to short-photoperiod induces hypoprolactinemia and decreases both the *in vivo* and *in vitro* testicular steroidogenic responses to hCG (3, 9), without affecting GH or IGF-I levels in male hamsters (23, 27).

Therefore, the present experiment was undertaken to compare the effects of increased GH levels in hyperprolactinemic hamsters versus those of decreased GH levels in hypoprolactinemic ones.

Materials and Methods

Adult (9 week old) male BIO F_1B Syrian hamsters *(Mesocricetus auratus)* from BioBreeders (Watertown, Mass), were housed in polycarbonate cages with filter tops, with free access to food and water, with controlled temperature (22 ± 2 °C) and short photoperiod (SPP) illumination (< 12.5 h light/24 h).

Hamsters were divided into three groups of six each. The first group served as control, and animals received no treatment. The second group was injected s.c. with 0.1 µg hGH/g twice a week for four weeks, then with 0.11 µg hGH/g twice a week for another two weeks and, finally, with 0.12 µg hGH/g twice a week during the last two weeks. The third group was injected s.c. with 2.5 µg GHRIH/g twice a day for eight weeks.

At the end of the treatment period, hamsters were sacrificed by exsanguination under anesthesia (100 µg pentobarbital/g). Testes were collected, decapsulated, weighed, and two fragments of similar

size were obtained. These testis fragments were then incubated with either 0 or 12.5 mIU hCG/ml media for 4 h at 32 + 1 °C in Krebs-Ringer bicarbonate buffer (1 mg glucose/ml media) using a 95 % O_2:5 % CO_2 atmosphere (17, 41).

Incubation media testosterone and estradiol levels were determined by solid-phase radioimmunoassay using kits (Diagnostic Products Corporation, Los Angeles, Ca) with ^{125}I-steroid tracers, and anti-steroids antibody-coated polypropylene tubes. Because these kits use a standard curve based on human serum, parallelism between the standard curve and a curve made up by different volumes from a pool of Syrian hamster testes incubation media was determined and confirmed (testosterone: standard curve: slope (m) = -1.688, Y intercept (Y^I) = 0.425, and correlation coefficient (r) = -0.999; incubation media: m = -1.692, Y^I = 0.431, and r = -1.000; estradiol: standard curve: m = −1.831, Y^I = 4.047, and r = −1.000; incubation media: m = −1.846, Y^I = 4.055, and r = −1.000).

Data from the RIAs were obtained using the RIAPLOT and RIADOSE programs (4). Data were evaluated by two-way analysis of variance (ANOVA) using the SPSS-X software on an IBM mainframe (29). For the ANOVA, data were tested for normality of distribution by the Kolmogorov-Smirnov test and for homogeneity of variance by Barlett's test, and log- or square-root transformed as needed (29, 35).

Results

No significant differences in the basal testicular incubation media testosterone concentration were detected among the three groups of animals. Treatment *in vivo* with human somatotropin caused media testosterone concentrations to increase, in response to the exposure *in vitro* to hCG, to levels twice those observed in untreated hamsters (table I). Also, contrary to what was expected, treatment *in vivo* with somatostatin did not only not reduce the *in vitro* testosterone response to hCG, but actually a numerical increase in this response was observed in somatostatin-treated hamsters when compared to untreated animals (table I).

Table I. *Testicular incubation media testosterone and estradiol concentrations as functions of the* in vivo *and* in vitro *treatments.*
Values are expressed as mean ± SEM for the six testis fragments per point. Points with a letter in superscript in common are not significantly different (P > 0.05; Student-Newman-Keuls procedure of the multiple range test).

Treatment Group	Testosterone (pg/mg testis)	Estradiol (pg/g testis)
Control+saline	28.58±5.39^{a}	0.32±0.15^{x}
Control+hCG	113.44±22.71^{bc}	4.50±2.43^{xy}
hGH+saline	37.30±5.89^{a}	1.75±1.45^{x}
hGH+hCG	276.12±131^{d}	13.76±7.10^{yz}
GHRIH+saline	19.28±4.18^{a}	0.80±0.51^{x}
GHRIH+hCG	189.51±47.57^{cd}	11.72±3.56^{z}

No significant differences in the basal testicular incubation media estradiol concentration were detected among the three groups of animals. Treatment *in vivo* with either human somatotropin or somatostatin increased media estradiol concentrations. Again, contrary to what was expected, treatment *in vivo* with somatostatin had a stimulatory effect on the estradiol response to hCG (table I).

Discussion

The working hypothesis for the present experiment assumed that, in hamsters with elevated somatotropic and lactotropic activity (hGH-treated), testicular function would be stimulated as observed in hGH transgenic mice (6). It also assumed

that, in hamsters with reduced somatotropic and lactotropic activity (GHRIH-treated SPP animals), testicular function would be impaired at least due to the reduction of PRL (3, 9). In these animals, the reduction of GH was expected to further impair gonadal function based on data obtained from PRL/GH deficient *dw/dw* and *df/df* mice, from GHRH receptor deficient *lit/lit* mice, and from IGF unresponsive *pg/pg* mice. In all of these models, which have deficient somatotropic axes, testicular steroidogenesis was impaired (1, 2, 7). Also, passive immunization against GHRH, delayed puberty in rats (8).

The present results do support the first hypothesis that increased somatotropic and lactotropic activity potentiates testicular steroidogenesis. Although, basal testosterone and estradiol productions were not affected, hCG-stimulated synthesis was greatly potentiated, especially where testosterone was concerned. This effect of hGH is probably due to the combined stimulatory effects of high PRL (5), of the GH-induced increase in IGF-I synthesized by the liver (21, 24, 25, 32, 36), and of the direct gonadotropic action of GH (11, 21, 34, 39).

Our second hypothesis was not supported by the present results. Since SPP hamsters have reduced PRL levels, and treatment with GHRIH reduces GH levels, there should have been impaired testicular steroidogenesis. However, our SPP GHRIH-treated hamsters had not only a statistically normal steroidogenic response to hCG, but it was numerically better for testosterone, and statistically better for estradiol, when compared to control animals. Therefore, a factor in these hamsters must have counteracted the effects of the lack of GH. Since GHRIH has been shown to be produced by Leydig cells, and treatment with GHRIH was the only variable introduced in this group of animals, it would be safe to propose that in GHRIH-treated hamsters, GHRIH in addition to decreasing GH levels, is capable of having a direct stimulatory or protective effect on the Leydig cell. The present results indicate a direct effect of GHRIH on testosterone synthesis, and its effect on estradiol synthesis is probably a result of this. However a direct effect of GHRIH on aromatase could not be discounted since the combined exposure to GHRIH and hCG appeared to increase the efficiency of aromatase.

Since GHRIH is produced by the Leydig cell (30, 33), and other members of the somatotrophic axis are also produced in the testis (10-12, 14, 16, 24-26, 31, 37, 40) it could be that GHRIH also plays a role in regulating gonadal function. Thus, we propose that Leydig cell GHRIH is part of a group of autocrine/paracrine factors that support steroidogenesis, and that, like other members of the somatotrophic axis (GHRH, GH and IGF-I) it is a positive regulator of Leydig cell function (14).

Acknowledgements

We thank Drs. A. Taraben, J. Jones and P. Gill, and Mrs. Caroline Lopez for their help in obtaining the samples. We thank Dr. E. J. Moticka, Ms. C. M. Herndon, and Ms. E. J. Edwards for their support. These studies were supported by NIH grant RR03295 (SSO), the SIU-OB/GYN Research Fund (SSO, AGA), and the ReproGen Research Fund (AGA).

S. S. OH, R. KHARDORI, D. K. KOPPLIN y A. G. AMADOR. *Efectos de la somatostatina y de la somatotropina sobre la esteroidogénesis testicular* in vitro *de hámster.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 51 (4) 187-192, 1995.

Se estudian en hámster, bajo un régimen de fotoperíodo corto, los efectos de inyecciones de somatotropina (GH), somatostatina

(GHRIH) o solución salina, durante ocho semanas. Posteriormente se incuban fragmentos testiculares en presencia o ausencia de hCG. Los niveles basales de testosterona y estradiol no varían significativamente en el medio de ninguno de los grupos. El tratamiento con GH potencia el efecto de la hCG sobre los niveles de testosterona en el medio. El tratamiento con GHRIH no inhibe el efecto de la hCG, como se esperaba, si no que tiende a producir aumento numérico en los niveles de testosterona. El tratamiento con GHRIH potencia el efecto de la hCG sobre los niveles de estradiol en el medio, mientras que la GH sólo produce un aumento numérico en los niveles de estradiol. La combinación de GHRIH y hCG parece aumentar la eficacia de la aromatasa testicular. Dado que datos publicados anteriormente indican que la deficiencia combinada de las acciones somatotrópicas y lactotrópicas inhiben severamente la esteroidogénesis testicular, el tratamiento con GHRIH debería haber tenido un efecto negativo aún mayor sobre la esteroidogénesis en hámsters expuestos a un fotoperíodo corto. Los resultados aquí presentados demuestran lo contrario, por lo que se postula que la GHRIH puede estimular directamente la esteroidogénesis testicular o, cuando menos, ejercer una acción protectora.

Palabras clave: Testículo, Somatostatina, Somatotropina, hCG, Hámster sirio, Testosterona, Estradiol.

References

1. Amador, A. G. and Bartke, A. (1982): *J. Endocrinol.*, 95, 301-309.
2. Amador, A. G., Bartke, A., Beamer, W. G. and Siler-Khodr, T. M. (1988): *Endocrinol. Exp.*, 22, 87-97.
3. Amador, A. G., Bartke, A., Klemcke, H. G., Siler-Khodr, T. M. and Stallings, M. H. (1985): *J. Reprod. Fertil.*, 74, 693-700.
4. Amador, A. G. and Hodges, S. L.(1989): *Comput. Biol. Med.*, 19, 343-351.
5. Amador, A. G., Klemcke, H. G., Bartke, A., Soares, M. J. and Siler-Khodr, T. M. and Talamantes, F. (1985): *J. Reprod. Fert.*, 73, 483-489.
6. Amador, A. G., Mayerhofer, A., Bartke, A. and Steger, R. W. (1989): *Mouse News Lett.*, 83, 183.
7. Amador, A. G., Parkening, T. A., Beamer, W. G., Bartke, A. and Collins, T. J. (1986): *Endocrinol. Exp.*, 20, 349-358.
8. Arsenijevic, Y., Wehrenberg, W. B., Conz, A., Eshkol, A., Sizonenko, P. C. and Aubert, M. L. (1989): *Endocrinology*, 124, 3050-3059.
9. Bartke, A., Matt, K. S., Amador, A. G., Klemcke, H.G., Brown, D., Gonzales D. and Hogan, M.P. (1985): *Int. J. Androl.*, 8, 232-242.
10. Berry, S. A. and Pescovitz, O. H. (1988): *Endocrinology*, 123, 661-663.
11. Berry, S. A. and Pescovitz, O. H. (1990): *Endocrinology*, 127, 1404-1411.
12. Berry, S. A., Srivastava, C. H., Rubin, L. R., Phipps, W. R. and Pescovitz, O. H. (1992): *Endocrinology*, 75, 281-284.
13. Ciampani, T., Fabbri, A., Isidori, A. and Dufau, M. L. (1992): *Endocrinology*, 130 (suppl.), 447.
14. Ciampani, T., Fabbri, A., Isidori, A. and Dufau, M. L. (1992): *Endocrinology*, 131, 2785-2792.
15. Closset, J., Dombrowicz, D., van den Broeck, M. and Hennen, G. (1991): *Growth Regulat.*, 1, 29-37.
16. Dombrowicz, D., Hooghe-Peters, E. L., Gothot, A., Sente, B., van Haelst, L., Closset, J. and Hennen, G. (1982): *Arch. Int. Physiol. Biochim. Biophys.*, 100, 303-308, 1992.
17. Dufau, M. L., Catt, K. J. and Tsuruhara, T. (1971): *Biochim. Biophys. Acta*, 272, 574-579.
18. Forsyth, I. A., Folley, S. J. and Chadwick, A. (1965): *J. Endocrinol.*, 31, 115-126.
19. Hartree, A. S., Kovacic, N. and Thomas, M. (1965): *J. Endocrinol.*, 33, 249-258, 1965.
20. Isaksson, O. G. P., Jansson, J.-O. and Gause, I. A. M. (1982): *Science*, 216, 1237-1238.
21. Kasson, B. G. and Hsueh, A. J. W. (1987): *Molec. Cell. Endocrinol.*, 52, 27-34.
22. Kleinberg, D. I. and Todd, J. (1980): *J. Clin. Endocrinol. Metab.*, 51, 1009-1013.
23. Klemcke, H. G., Bartke, A. and Borer, K. T. (1983): *Biol. Reprod.*, 29, 605-614, 1983.
24. Lin, T., Haskell, H., Vinson, N. and Isgaard, J. (1986): *Biochem. Biophys. Res. Commun.*, 137, 950-956.
25. Lin, T., Haskell, H. and Vinson, N. (1986): *Endocrinology*, 119, 1641-1647.
26. Lin, T., Wang, D., Calkins, H., Guo, H., Chi, R. and Housley, P. R. (1990): *Mol. Cell Endocrinol.*, 73, 147-152.
27. Meijers, M., Woutersen, R. A., van Garderen-Hoetmer, A., Bakker, G. H., de Jong, F. H., Foekens, J. A and Klijn, J. G. M. (1991): *Carcinogenesis*, 12, 1707-1713.
28. Moretti, C., Fabbri, A., Gnessi, L., Bonifacio, V., Bolotti, M., Arizzi, M., Nazzicone, Q. and Spera, G. (1990): *J. Endocrinol. Invest.*, 13, 301-305.

29. Norusis, M. J. (1987): The SPSS Guide to Data Analysis for SPSS-X. Chicago:SPSS Inc.
30. Pekary, A. E., Yamada, T., Sharp, B., Bhasin, S., Swerdloff, R. S. and Hershman, J. M. (1984): *Life Sci.*, 34, 939-945.
31. Pescovitz, O. H., Berry, S. A., Laudon, M., Ben-Jonathan, N., Martin-Myers, A., Hsu, S.-M., Lambros, T. J. and Felix, A. M. (1990): *Endocrinology*, 127, 2336-2342.
32. Saez, J. M., Avallet, O., Naville, D., Perrard-Sapori, M. H. and Chatelain, P. G. (1989): *Ann. N. Y. Acad. Sci.*, 564, 210-231.
33. Sasaki, A. and Yoshinaga, K. (1989): *J. Clin. Endocr. Metab.*, 68, 996-999.
34. Singh, H., Griffith, R. W., Takahashi, A., Kawauchi, H., Thomas, P. and Stegeman, J. J. (1988): *Gen. Comp. Endocrinol.*, 72, 144-153.
35. Sokal, R. R. and Rohlf, F. J. (1981): Biometry (2nd), W. H. Freeman and Co., San Francisco, Ca
36. Spiteri-Grech, J. and Nieschlag, E. (1992): *Horm. Res.*, 38 (suppl. 1), 22-27.
37. Srivastava, C. H., Collard, M. W., Rothrock, J. K., Peredo, M. J., Berry, S. A. and Pescovitz, J. K. (1993): *Endocrinology*, 133, 83-89.
38. Suhr, S. T., Rahal, J. O. and Mayo, K. E. (1989): *Molec. Endocrinol.*, 3, 1693-1700.
39. Thorlacius-Ussing, O, Flyvberg, A., Joergensen, K. D. and Oerskov, H. (1988): *Acta Endocrinol.* (kbh), 117, 65-72.
40. Tsagarakis, S., Ge, F., Besser, G. M. and Grossman, A. (1991): *Life Sci.*, 49, 1627-1634.
41. Wolfe, H. G., Bartke, A., Amador, A., Van Sickle, M., Dalterio S. and Brown, D. (1983): *J. Endocrinol.*, 90, 367-373.