EUROP. J. OBSTET. GYNEC. REPROD. BIOL., 6/3, 115–119. EXCERPTA MEDICA

Biological and immunological studies of a placental gonadotropic protein*

M.A. Castellano, S. Galimidi, C. Míguez-Wensko, A. Méndez-Tula, J.A. Guisantes, Y. Sardi-Valverde and J. Brovetto-Cruz

Laboratory of Protein Hormones, Latin American Center of Perinatology and Human Development (PAHO/WHO), Casilla de correo 627, Montevideo, Uruguay

CASTELLANO, M.A., GALIMIDI, S., MÍGUEZ-WENSKO, C., MÉNDEZ-TULA, A., GUISANTES, J.A., SARDI-VALVERDE, Y. and BROVETTO-CRUZ, J. (1976): Biological and immunological studies of a placental gonadotropic protein. *Europ. J. Obstet. Gynec. reprod. Biol., 6/3,* 115–119.

Precocious sexual maturity, ovarian weight increase and stimulation of the sex accessory organs in impuber female mice, was induced by a partially purified protein fraction obtained from human placenta. The relationship between this biologically active protein and HCG was studied by a comparative immuno-double diffusion technique (Ouchterlony) and two agglutination inhibition tests. Results showed that this protein with gonadotropic activity is neither immunologically related to HCG nor contaminated with this hormone.

gonadotropins; placenta

Introduction

The presence of an uterotropic substance in human placental extracts has been reported (Beas and Flores, 1969; Sardi-Valverde, Míguez-Wensko, Castellano, Ucar and Brovetto-Cruz, 1976; Brovetto-Cruz, Míguez-Wensko, Sardi-Valverde, Ucar, Guisantes, Galimidi and Castellano, 1976), and the protein nature of this active substance has been established (Brovetto-Cruz et al., 1976). Furthermore, the study of the uterotropic activity of a partially purified protein fraction from this extract, showed no activity in spayed rodents, suggesting the gonadotropic property of the placental preparation (Brovetto-Cruz et al., 1976).

In this paper we study other biological properties of this extract that support its assumed gonadotropic activity. The immunological relationship between our protein fraction and HCG and the possible contamination with this hormone, were also studied using the immuno-double diffusion technique and agglutination inhibition tests for HCG.

* This paper has been partially supported by research grant 6.8.1.75 from the Progama Latinoamericano de Investigaciones en Reproducción Humana (PLAMIRH).

Material and methods

Biological studies

The experiments were performed in randomly bred, Swiss albino mice from a hybrid colony. Ten immature mice, 20 days old, weighing 9 ± 1 g, were injected subcutaneously with 0.5 mg of the active preparation (F-II), from the 20th day of life until they opened vagina. For control, a second group of 9 mice was treated in the same manner with saline. 24 h after opening of vagina, the animals were sacrificed

by cervical dislocation and the genital apparatus excised. The uteri were expressed on filter paper and the wet uterine weight determined on a precision balance. The ovaries were dried on filter paper and weighed.

The Fallopian tubes were examined with transparent light under a stereomicroscope to detect ovulation. This examination showed failure of the tube to balloon. The ovaries, tubes and vagina were then fixed, embedded and processed for histological study with PAS-hematoxylin.

In addition, a dose–response curve for uterine weight was prepared to determine the minimal dose of F-II necessary to induce a significant increase of the uterine weight. Immature female mice, weighing 9–11 g at 21 days of age, were used. The dose–response curve for uterine weight was prepared using the following daily doses of F-II: 50, 100, 150, 250, 350 and 500 μg. The animals were injected subcutaneously once a day for 3 days with 0.1 ml of the corresponding dose dissolved in saline. On the 4th day the mice were killed and the uteri were cleaned, blotted on filter paper and weighed. 7 mice were used for each dose level.

Immunological studies

Immunodiffusion. The comparative double diffusion (Ouchterlony) of HCG * (100 IU/ml) and F-II (10 mg/ml) against anti-HCG serum was performed. The rabbit anti-HCG serum absorbed with human normal serum, used in this technique, was prepared using a commercial preparation as antigen (Progon Dispert).

Agglutination inhibition tests. An F-II solution of 21 mg/ml in pH 4.5, 0.3 M sodium acetate buffer was analyzed by the agglutination inhibition slide test, using latex particles coated with HCG (Pregnosticon, Organon).

With the same preparation of F-II, the hemagglutination inhibition assay for HCG was performed according to the technique described by Wide (1962) without modifications. As standard, three commercially available chorionic gonadotropins (Endocorion, Elea; Primogonyl, Schering, and Progon, Dispert) were used. Standard solutions of 5 IU/ml in 0.15 M phosphate buffer pH 6.4 were prepared for each commercial hormone. A 0.5 mg/ml solution of F-II in the same buffer was used. All these solutions were assayed in parallel using the same suspension of HCG cells and the same antiserum. All specimens were serially two-fold diluted in 10 tubes containing 0.15 M phosphate buffer pH 6.4. As control, tubes containing antiserum and no HCG, as well as tubes that only contained HCG or F-II solutions were used.

* (Primogonyl, Schering).

Results

A very precocious opening of vagina was obtained when F-II was administered to immature mice (see Table I). Also, the ovarian weight of the F-II treated mice was significantly augmented as compared with that of the puberal control group. This ovarian weight increase could be essentially attributed to an important augmentation in the number of medium-sized follicles as can be evaluated by the simple histological examination. Young corpora lutea and large follicles were also seen. A marked increase in uterine weight was produced by F-II (see Table I).

Vaginal histology demonstrates that F-II stimulates this organ, inducing proliferation of its epithelium (Fig. 1, A). 7 of 10 animals showed a conspicuous proliferation and keratinization of vaginal epithelium, while the others showed an intense mucification.

Although failure of the tube to balloon was observed in the stereomicroscope and the search for ova by this procedure was negative, microscopic exploration of the oviduct in histological sections showed that 3 of 10 animals had tubal eggs (see Fig. 1, C). In the control group, 2 of 9 animals showed tubal eggs. The histological study of ovaries showed young corpora lutea in the ovulating mice, while medium-sized follicles and large follicles were seen in the remaining animals. It should be pointed out that the F-II treated animals ovulated an average of 10 days earlier than control animals (see Table I).

The study of the dose–response curve for the uterotropic activity of our placental preparation showed that the response is dose dependent (Fig. 2), and that 50 μg is capable to induce an increase in uterine weight of approximately 100%.

TABLE I Effects of F-II on the reproductive organs of immature female mice

Animals	Treatment	Age at vaginal opening (days ± SE)	With tubal eggs	Ovarian wt (mg ± SE)	Uterine weight (mg ± SE)	
					wet wt	dry wt
Immature mice	F-II	23.5 ± 0.2*	3	10.2 ± 1.2*	48.3 ± 7.8*	11.0 ± 1.7*
Immature mice	saline	33.1 ± 0.6	2	4.0 ± 0.2	10.6 ± 1.1	4.6 ± 0.4

* Differences between the means of F-II treated and control groups were statistically significant at 0.5%

Immunological studies

The comparative double diffusion of HCG and F-II against rabbit anti-HCG serum showed a precipitation line with HCG, while no reaction was observed with F-II (Fig. 3).

The agglutination inhibition slide test using latex particles coated with HCG showed that a F-II solution did not inhibit the agglutination of the latex particles, while a 3 to 5 IU/ml solution of HCG inhibits it.

In the hemagglutination inhibition reaction, all the tubes containing F-II gave a clear positive image of hemagglutination. The least amount of HCG detectable with this technique in our laboratory was 0.2–0.3 IU/ml. Therefore if HCG is present in F-II its amount would be lower than 0.2–0.3 IU in 0.5 mg of the placental preparation, contained in the first tube of the serial dilution of F-II.

Discussion

Results presented here show that our placental preparation possesses other biological properties besides its uterotropic activity. It produces a stimulating effect on the vagina shown by proliferation of its epithelium accompanied by keratinization or intense mucification in the immature female mouse. Also, it produces in this animal an outstanding increase of the ovarian weight associated with a precocious opening of vagina and ovulation that occurs an average of 10 days before that of the control animals.

The increase of the ovarian weight accompanied by ovulation and precocious sexual maturity indicates that this placental protein fraction has gonadotropic activities. This assumption is also supported by our results on the effect of this preparation in spayed animals (Brovetto-Cruz et al., 1976) showing the absence of uterotropic activity in castrated female mice.

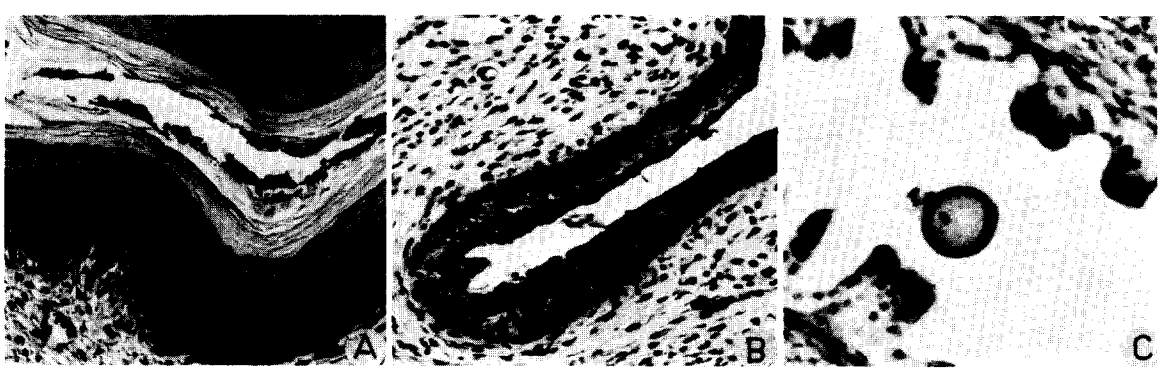

Fig. 1. A: Vaginal mucosa of a F-II treated immature mouse; note a conspicuous proliferation and cornification of the epithelium; ×175.

B: Vaginal mucosa of an immature control animal sacrificed at 23 days of life (when the F-II treated animals open vagina); ×190.

C: Uterine tube of F-II treated mouse; there is one ovulated egg; ×190.

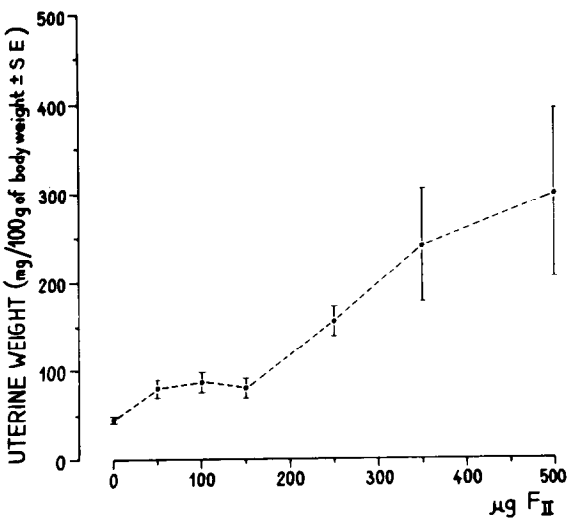

Fig. 2. Dose–response curve for the uterotropic activity of F-II in immature female mice.

These gonadotropic properties may explain the already reported uterotropic activity and the vaginal stimulation here described.

The most striking results of our paper are that three immunological techniques showed no identity between our gonadotropic placental preparation and HCG. The comparative immuno-double diffusion of HCG and F-II showed that our placental protein fraction is different from HCG. However, this result does not discard that our preparation might be contaminated with HCG, and that this contamination would explain the gonadotropic activity that we demonstrated. In this sense, the agglutination inhibition tests showed that contamination with HCG, if it exists, must be lower than 0.4–0.6 IU per mg of F-II. We therefore conclude that in the minimal active dose of F-II (50 µg) the HCG contamination would be lower than 0.02–0.03 IU. This amount of HCG cannot explain the biological activity of our preparation, since a dose of approximately 0.25 IU of HCG would be necessary to induce a 100% increase in the uterine weight of the mouse (Bell, 1968).

Finally, results obtained by immunological techniques showed that this placental protein is immunochemically different from HCG. However, the existence of biologically active HCG molecules with low immunological activity has been reported (Matties and Diczfalusy, 1971). Nevertheless, this was found only in HCG obtained from pregnancy urines, while in both serum and placental extracts there is coincidence of immunological and biological activities. Therefore, if all the biologically active HCG obtained from the placenta were also immunologically reactive (Matties and Diczfalusy, 1971), our protein fraction would be a gonadotropic placental protein different from HCG.

Our evidence is not enough to postulate the existence in human placenta of a gonadotropic hormone different from HCG, but is suggests that this parenchyma contains a gonadotropic protein at least immunologically different from HCG.

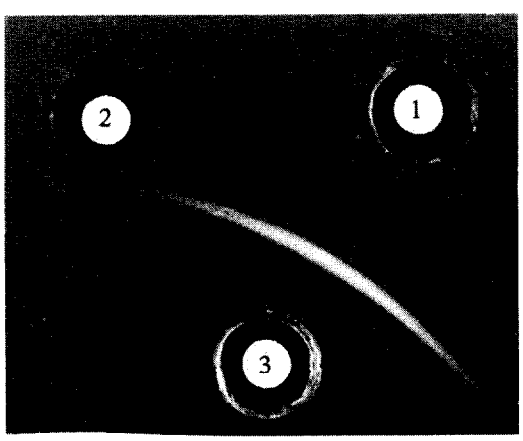

Fig. 3. Comparative immuno-double diffusion technique of HCG and F-II against anti-HCG serum. 1: HCG 100 IU/ml; 2: F-II 20 mg/ml; 3: anti-HCG serum.

Acknowledgement

We are grateful to Prof. R. Caldeyro-Barcia for his encouragement and advice. We wish to acknowledge with thanks the skillful technical assistance of Mrs. Alba Berro-Campelo.

References

Beas, F. and Flores, H. (1969): Characterization and properties of a new placental protein. *Nature (Lond.), 221,* 574.

Bell, E.T. (1968): Studies on uterine growth in immature mice. In: *Gonadotropins, Proc. of the Workshop Conference, Mexico, 1968.* Editor E. Rosemberg. Geron-X Inc., Los Altos, Calif.

Brovetto-Cruz, J., Míguez-Wensko, C., Sardi-Valverde, Y., Ucar, D., Guisantes, J.A., Galimidi, S. and Castellano, M.A. (1976): Chemical and biological characterization of an active substance of the human placenta. *Europ. J. Obstet. Gynec. reprod. Biol., 6,* 109.

Matties, D.L. and Diczfalusy, E. (1971): Relationship between physico-chemical, immunological and biological properties of human chorionic gonadotrophin. 1. Properties of human chorionic gonadotrophin as found in tissues and body fluids. *Acta endocr. (Kbh.), 67,* 434.

Sardi-Valverde, Y., Míguez-Wensko, C., Castellano, M.A., Ucar, D. and Brovetto-Cruz, J. (1976): Attempts of estrogen extraction from human placental uterotropic preparations. *Int. Res. Commun. Syst., med. Sci.,* submitted for publication.

Wide, L. (1962): An immunological method for the assay of human chorionic gonadotrophin. *Acta endocr. (Kbh.), Suppl. 70,* 1.