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Chromatographic Studies on a Chorionic Gonadotropic Activity in the Placenta of the Rat, Mouse and Hamster

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ABSTRACT

Acetone-ether extracts of rat, mouse and hamster placentae were fractionated by molecular sieve chromatography on Sephadex G-200. The fractions were tested for immunoreactivity in human chorionic gonadotropin (hCG), hCG-beta-subunit and alpha-subunit radio-immunoassay systems. The elution profiles were compared with those of similar chromatographic studies of a human placental extract and of purified preparations of hCG and its subunits. The results indicate that rodent placentae have a chorionic gonadotropin and that this hormone in the rat, mouse and hamster is structurally similar to hCG with its alpha- and beta-subunits. Extracts of rat and hamster placentae had a gonadotropic activity similar in concentration to that found in normal human placentae at term. Until now, it has been difficult to find an animal model for studying how the production of chorionic gonadotropin is regulated. Our results suggest that rodents may be suitable for such an investigation.

INTRODUCTION

Very little is known about the regulation of the production of placental chorionic gonadotropin. One reason for this lack of knowledge is that it has been difficult to find an animal model for such studies. In fact, the presence of a placental gonadotropin in rodents has been a matter of controversy. Some workers (2, 4, 10) found evidence for a rat placental luteotropin, while others (3, 8) were unable to detect gonadotropins in the rat placenta. It has been considered that the only detectable gonadotropin in the rat placenta has both luteotropic and mammotropic activity (11). However, studies on rat chorionic mammotropin suggest that the lactogenic and the luteotropic activities derive from two different entities (9).

In a recent investigation (14), extracts of placentae from the rat, mouse and hamster were found to contain components active in radioimmunoassays for human chorionic gonadotropin (hCG) and its alpha- and beta-subunits. To study the nature of these activities the extracts were tested after molecular sieve chromatography and the elution patterns were compared with those from similar chromatographic studies of a human placental extract and of purified preparations of hCG and its subunits.

MATERIAL AND METHODS

Placental tissue

Seventeen rat placentae were collected on day 18 of pregnancy, 42 hamster placentae were collected on day 15 and 16 of pregnancy, and 64 mouse placentae were collected on day 15 of pregnancy. The average weights of the placenta from the rat, mouse and hamster were 437 mg, 68 mg, and 304 mg, respectively. A human term placenta weighing 488 g was obtained from a woman who delivered a healthy female child. Except for the mouse placentae, which were frozen and stored at −20°C until extracted, all other placentae were put into ice-cold acetone and extracted immediately.

Extraction method

The cord and membranes were dissected from all placentae before they were homogenized in ice cold acetone 5 ml/g plus ice cold either 1 ml/g. Tissue extracts were kept at −4°C overnight and were collected either by filtration on a Buchner funnel (human placental tissue) or by centrifugation in the case of the rodent material. The tissue extracts were washed in acetone and ether after which the drying was completed in a desiccator. The completeness of the extraction method for the removal of steroid material was checked in ovariectomized oestrone primed mice (5). The extract had no activity in this test.

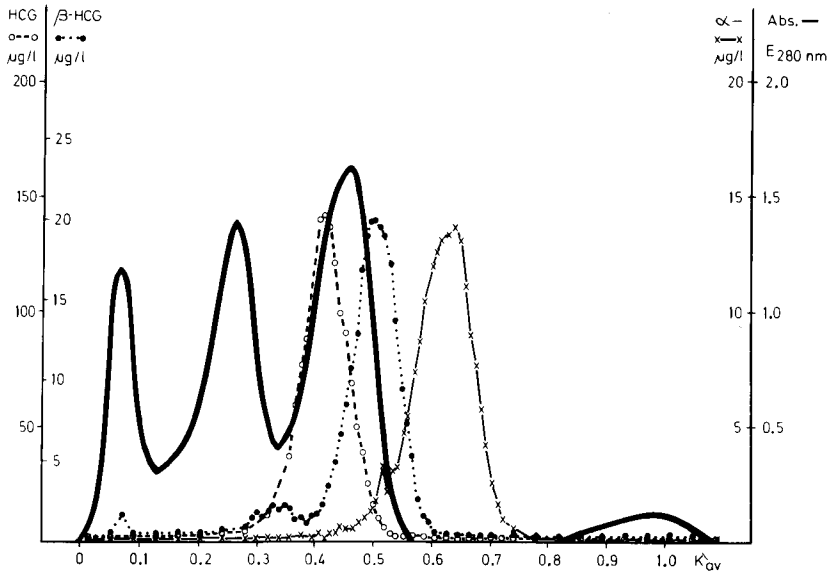

Fig. 1. Elution patterns for purified hCG, hCG-beta-subunit and hTSH-alpha-subunit preparations added to 3 ml samples of normal human male serum and chromatographed on Sephadex G-200. The mean curve for optical density at 280 nm from the three chromatographic studies is indicated. The peak at K_{av} 0.45 is serum albumin.

Chromatography

A 26×930 mm Sephadex G 200 column was equilibrated with 0.1 M Tris-HCl buffer of pH 7.5 with 0.2 M NaCl and that buffer was used for all subsequent chromatographic studies. The flow was against gravity with a rate of 9 ml per h, and 3 ml fractions were collected.

The degree of retardation of immunoreactive material on the column was expressed in K_{av} values. The following materials were fractionated: an extract of 350 mg of rat placental tissue, an extract of 456 mg of hamster placental tissue, an extract of 1440 mg of mouse placental tissue, an extract of 480 mg of human term placental tissue, and 3 ml samples of a normal human male serum to which a purified preparation hCG, hCG-beta-subunit or hTSH-alpha-subunit had been added.

Bioassay

The gonadotropic activity of the extracts of rat and hamster placentae was assayed by using the increase in uterine weight of 24-day-old mice (Swiss albino strain) as the index of response (6). The extracts were disintegrated in 0.9% NaCl solution in a high-speed tissue grinder before being assayed. The WHO 2nd International Standard preparation of hCG was used as a standard and the assay design was 2×2 with 5 mice at each dose level for standard and unknown.

Radioimmunoassay

The immunoreactivities of the extracts and different fractions from the chromatographic separations were assayed by solid phase radioimmunoassays RIA (13) for hCG and for the alpha- and beta-subunits of this hormone. In the hCG assay, the reagents were ^{125}I-labelled hCG and anti-hCG, and in the beta-hCG assay ^{125}I-labelled beta-hCG and anti-beta-hCG. In the alpha-subunit assay, the ^{125}I-labelled antigen was hLH-alpha-subunit and the antibody was against hTSH-alpha-subunit. In this system, the alpha subunits of hLH, hTSH, hFSH, and hCG have a similar activity. The subunit preparations and their antisera were supplied by the N.I.A.M.D.D., National Institutes of Health, Bethesda. The immunoreactivities were expressed in µg/l using the highly purified preparations as reference standards.

RESULTS

Both the rat and the hamster placental extracts had a significant activity in the bioassay. The gonadotropic activity was equivalent to 5.77 IU per placenta or 13.2 (95% fiducial limits: 11.3–15.2) IU per g of placental tissue for the rat and the corresponding figures for the hamster were 0.82 IU or 2.72 (95% fiducial limits: 1.9–3.7) IU, respectively.

The extracts of rat, mouse and hamster placentae had a significant activity in the three RIA systems. To study the nature of these activities, all the extracts were fractionated by molecular sieve chromatography on Sephadex G-200 and the fractions were collected and tested for immunoreactivity in the three RIA systems. For comparison, an acetone-ether extract of human term placenta was examined in exactly the same way as those of the rodent placentae. Furthermore, the elution patterns for purified hCG, hCG-beta-subunit and TSH-alpha-subunit preparations added to 3 ml samples of normal human male serum were also investigated using the same size of column for chromatography.

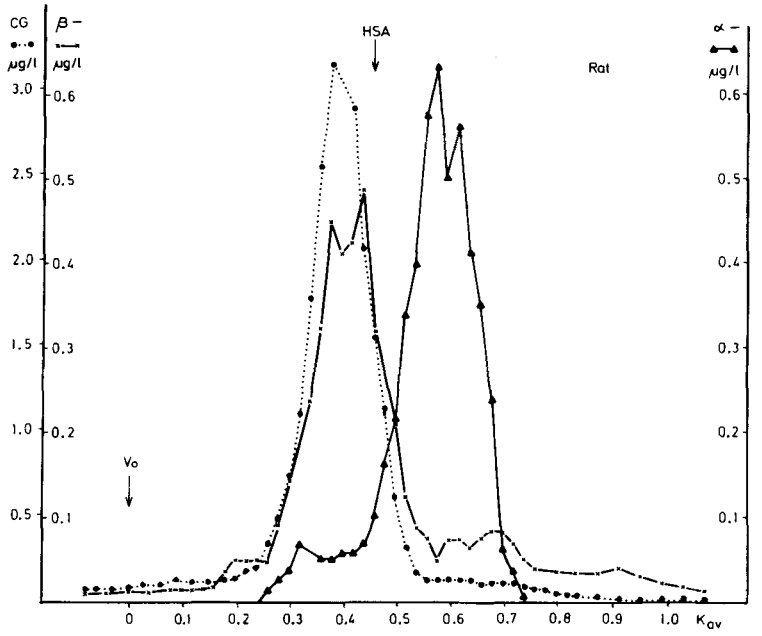

Fig. 2. Elution profile of an extract of 350 mg of rat placental tissue, equivalent to 0.8 of a placenta, chromatographed on Sephadex G-200. Each fraction was assayed in hCG, hCG-beta-subunit and alpha-subunit radioimmunoassays. The position for human serum albumin (HSA) is indicated.

The immunological activity of these three purified preparations plotted in relation to elution volume, K_{av} values, are shown in Fig. 1, which also shows the mean curve for the absorbance at 280 nm from the three chromatographic studies. The activities and elution profiles from the chromatographic separations of the different placental extracts are plotted in a similar manner and are shown in Figs. 2–5. In order to emphasize the positions of the components of the various placental extracts, the activities of the eluted material are not plotted on the same scale.

The activities detected in the extracts of rat, mouse and hamster placentae were found in frac-

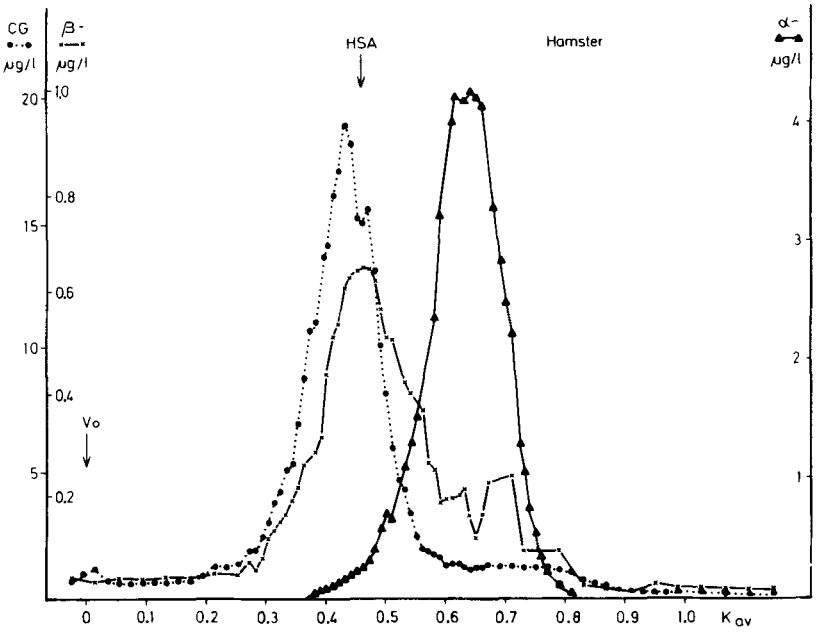

Fig. 3. Elution profile of an extract of 456 mg of hamster placental tissue, equivalent to 1.5 placentae, chromatographed on Sephadex G-200. Each fraction was assayed in hCG, hCG-beta-subunit and alpha-subunit radioimmunoassays. The position for human serum albumin (HSA) is indicated.

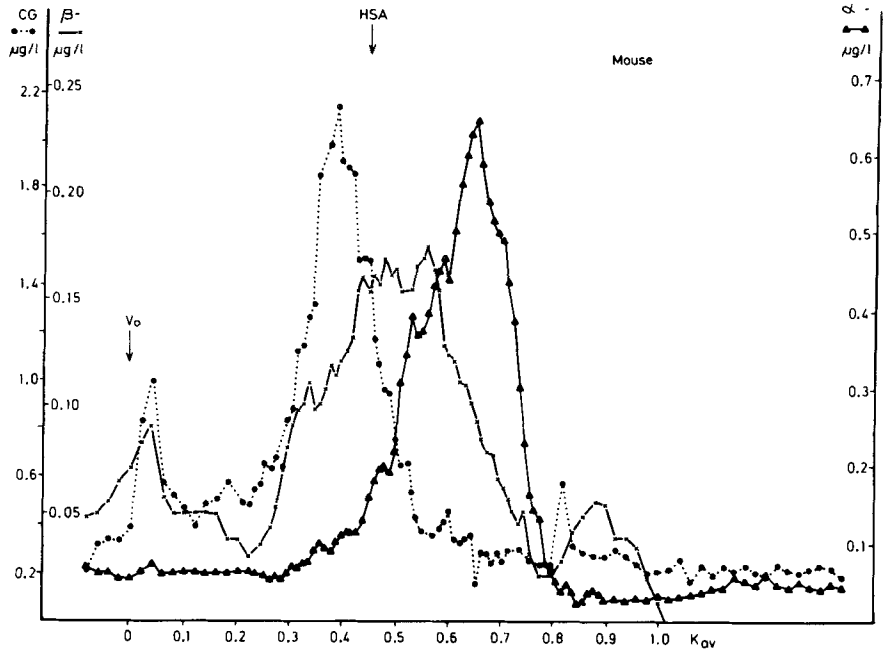

Fig. 4. Elution profile of an extract of 1440 mg of mouse placental tissue, equivalent to 21 placentae, chromatographed on Sephadex G-200. Each fraction was assayed in hCG, hCG-beta-subunit and alpha-subunit radioimmunoassays. The position for human serum albumin (HSA) is indicated.

tions eluted in the same positions as those of the human placental hCG, hCG-alpha-and hCG-beta-subunit activities. The K_{av} values for maximal CG, CG-beta-subunit, and alpha-subunit activities were around 0.40, 0.50, and 0.65, respectively. Dose response curves over a 10-fold dilution of eluted fractions having the highest activity, did not show a significant departure from parallelism when com-

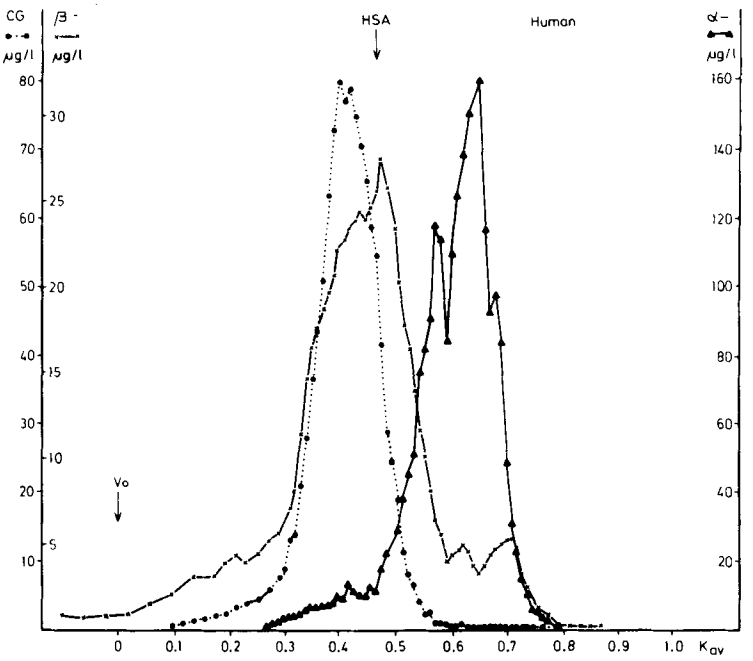

Fig. 5. Elution profile of an extract of 480 mg of human placental tissue, equivalent to 0.001 of a placenta, chromatographed on Sephadex G-200. Each fraction was assayed in hCG, hCH-beta-subunit and alpha-subunit radioimmunoassays. The position for human serum albumin (HSA) is indicated.

pared with the standard curves in the three systems. Using the hCG immunoassay, the activities eluted between K_{av} values 0.3 and 0.5 were equivalent to 295, 1018, and 72 ng of hCG per g of placenta tissue from the rat, hamster and mouse respectively. The corresponding value for the human was 4707 ng of hCG per g.

All placental extracts contained significant amounts of free alpha-subunit activities. The amount of this activity compared with the amount of CG activity was large in the human placental extract. With the beta-subunit assay the activities eluted by chromatography of the mouse, hamster and human placental extracts were found in the same position as that of free hCG-beta-subunits.

DISCUSSION

The concentration of gonadotropins found in the rat and hamster placentae was similar to that for the human placenta at term (range 2.9–42.4 IU/g) using the same bioassay method and hCG standard (7). When these extracts together with that of mouse placentae were assayed in radioimmunoassay systems for hCG-beta-unit and hCG-alpha-subunit they were found to have significant activities in all three systems. However, such an activity could be a non-specific inhibition of the immunological reaction. The chromatographic studies revealed that the immunological activities were eluted in similar positions when the extracts of rat, mouse and hamster placentae were compared with that of the human placenta. These results indicate structural similarities between the components in the four different placental extracts and further supports the suggestion (1, 4, 14) that the luteotropic principle in the rat placenta is chorionic gonadotropin. They also indicate that this hormone in the rat, mouse and hamster is structurally similar to hCG with its two subunits.

The degree of cross-reactivity between the human and the rodent material is not known and, therefore, the values estimated for CG immunological activity in the rodent placenta cannot be taken as absolute quantities. The finding of a large amount of free alpha-subunit activity compared with CG activity in the human placental extract is in agreement with earlier work (12). The results of the beta-subunit assays indicate that the extracts of human, hamster and mouse placentae contain free beta-subunit activities. However, hCG showed a considerable cross-reactivity in the hCG-beta-subunit assay. Because of this and the fact that the chromatographic system could not completely separate hCG from hCG-beta subunits, it was not possible to estimate the amount of 'free' beta-subunit activities in the different placental extracts.

In conclusion, the results of this investigation indicate that the rat, mouse, and hamster placenta produce a chorionic gonadotropin and that this hormone is structurally similar to hCG. Consequently, rodents may be suitable animals for studying the regulation of the production and release of chorionic gonadotropin from the placenta.

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