Upsala J Med Sci 86: 249–258, 1981

Electrophoretic and Gel Chromatographic Analyses of Follicle-Stimulating Hormone in Human Serum

Leif Wide

From the Department of Clinical Chemistry, University Hospital, Uppsala, Sweden

ABSTRACT

The pleomorphism of follicle-stimulating hormone (FSH) in human serum was investigated by gel chromatography on Sephadex G-200 and zone electrophoresis in agarose suspension. The FSH activity was measured by radioimmunoassay. By gel chromatography FSH in sera from 3 males and 5 females of various ages was eluted within a narrow range after albumin and no sex hormone related pleomorphism was found. Electrophoresis in 0.17% agarose suspension was found to be a technique suitable for analysis of differences in charge of FSH in sera from men and women. A total of 31 sera from 25 individuals was investigated. The results strongly indicate that there is in man a sex hormone related pleomorphism of FSH with less acidic forms of FSH in sera of normal women of fertile age than in sera of men and post-menopausal women. Qualitative analysis of FSH in serum by electrophoresis may become an important complement to the quantitative determinations of the FSH concentration for the clinical diagnosis of reproductive disorders and of FSH producing tumours.

INTRODUCTION

A pleomorphism of the follicle-stimulating hormone (FSH) extracted from pools of human pituitaries has been shown with respect to molecular charge and size (10,11). The physiological significance of such a pleomorphism of human FSH is not known. To some extent it may be due to transformations of FSH during the life cycle of the hormone: from pro-hormones, to hormone, to more or less degraded forms of the hormone. The latter forms may be normally occuring in the pituitary or induced due to the treatment of the material investigated.

Evidence for a sex hormone related pleomorphism of the pituitary FSH was presented some years ago from experiments in the rhesus monkey and in the rat (2, 3, 7, 8, 9). In both species the pleomorphism of pituitary FSH was revealed by gel chromatography. In the rhesus monkey the female FSH was of apparent larger molecular size than the male FSH and the largest forms of FSH were found in the

pituitaries of castrated animals. In the rat, the males had the largest forms and a smaller form appeared after orchiectomy. In this animal the smallest FSH was found in the female. In both animal species the smaller forms disappeared more rapidly from the circulation than the larger forms. These results indicate that the gonadal steroid feed-back effect may be not only quantitative but qualitative. However, the results also indicate that there are considerable species differences.

Evidence for a similar sex hormone related pleomorphism of human FSH has hitherto not been reported. Studies like those made on pituitaries from the rhesus monkey and the rat are naturally not easily made on human material. This paper describes electrophoretic and gelchromatographic analyses of FSH in human serum. The concentration of FSH in serum is low in men and in women of fertile age. Therefore separation techniques permitting analyses of 2 to 4 ml of serum were used in combination with extremely sensitive radioimmunoassays to detect FSH. The results indicate that with the use of zone electrophoresis in agarose suspension it is possible to demonstrate the presence of a more acidic 'male' form of FSH and a less acidic 'female' form of FSH in human serum.

MATERIAL AND METHODS

Serum specimens

Serum specimens obtained from 8 individuals were investigated by gel chromatography. The sex and age of the individuals are given in Table 1. A serum specimen was investigated both without and with the addition of purified FSH (12); 15 µg added to 3 ml of serum. One serum specimen was obtained 45 min after injection of 100 µg of luteinzing hormone-releasing hormone (LRH) to a healthy woman on day 14/28 of the menstrual cycle.

For the electrophoretic studies 31 serum specimens were obtained from 25 individuals (Table 2). Five of these serum specimens were obtained 30 to 45 min after LRH injection and two samples were obtained about 4 h after inhalation of the LRH-agonist D-Ser(TBU)^{6}-LRH used in a contraceptive study (1). Included in the study were serum specimens from a patient with an FSH producing pituitary adenoma (14, 15), from a patient with a premature menopause, and from a patient with a primary amenorrhoea (Table 2).

Gel chromatography

Three ml of the serum specimens were gel chromatographed on a 26 x 930 mm Sephadex G-200 Superfine (Pharmacia Fine Chemicals, Uppsala, Sweden) column, equilibrated with 0.1 M Tris-HCl buffer of pH 7.5 with 0.2 M NaCl. The flow was against gravity with a rate of 9 ml per h with 3 ml fractions collected. The

degree of retardation was expressed in K_{av}-values (6). The protein distribution in the eluate was determined by measuring the absorbance at 280 nm. The FSH and luteinizing hormone (LH) activities were determined by radioimmunoassay of 0.5, 1.0, or 1.5 ml aliquotes of the eluted fractions. The differences between K_{av}-values of the hormone peaks and the albumin peak was calculated for each serum specimen.

Zone electrophoresis in agarose suspension

Zone electrophoresis was performed in a column with 0.17 per cent agarose suspension in 0.075 M sodium veronal buffer of pH 8.6 according to the technique described by Hjertén (4). The agarose (Agarose C, Pharmacia Fine Chemicals, Uppsala, Sweden) was dissolved by boiling and left to cool for 2 - 4 h before the gel was broken by gentle stirring to form a suspension. Before the electrophoresis the serum specimen was gel chromatographed on a Sephadex G-25 column using the 0.075 M sodium veronal buffer for equilibration and elution. By this procedure the protein fraction of the serum was diluted 1.5 times. The eluate was mixed with ^{125}I-labelled human LH, FSH and albumin and with the agarose pellet, from a volume of the suspension corresponding to 1 cm of the column, and then applied to the column as described (4).

Columns of three different sizes were used: column A: 1.3 x 66, column B: 2.0 x 67 cm and column C 2.8 x 67 cm. The following conditions were used for column A (figures for columns B and C are given within parentheses): Electrophoresis of 0.5 ml (B: 2 ml; C: 4 ml) of serum was performed at a voltage of 1000 V (B: 1200 V; C: 800 V) and a current of 50 mA (B: 100 mA; C: 40 mA) for about 20 h (B: 20 h; C: 36 h). After electrophoresis a funnel fitted with a polyvinyl chloride tubing to a sample collector was introduced from the top of the electrophoresis tube down to the agarose suspension. To simplify the procedure of emptying the electrophoresis column stopcocks were fitted to the top of the shunt column and the ground glass joint was placed straight above the electrophoresis column. The latter arrangement made it easy to introduce the funnel with the plastic tubing on the top of the agarose suspension. A peristaltic pump attached to the top of the shunt tube was started pumping buffer solution into the tube. The fractions collected were 1.1 ml (B: 2.2 ml; C: 3.3 ml) and the agarose suspension was pumped out during 1 h (B: 2 h; C: 3 h). The agarose was removed by centrifugation at 2,400 x g for about 20 min. The supernatant (A: 0.6 ml; B: 1.3 ml; C: 1.8 ml) could be used without further treatment for the analyses by radioimmunoassay. The protein distribution could be assayed without removal of the agarose by measuring the difference in absobance at 284 nm and 310 nm (5). The migration rate of ^{125}I-labelled FSH in relation to albumin was calculated for each run and used as a control of reproducibility of the electrophoresis.

Hormone assays

FSH and LH in serum and in fractions from gel chromatography and FSH in fractions from electrophoresis were measured by a radioimmunosorbent technique as described previously (16). The sensitivity of the assays was adjusted by varying the amount of the test material analysed from 0.1 ml to 1.5 ml and by varying the time of incubation before the labelled antigen was added from 2 h to 6 days. The maximal sensitivity of the FSH and LH assays was about 1 and 4 pg, respectively. Purified FSH (12) and LH (13) preparations used both for labelling and as standards were supplied by Dr. P. Roos, Institute of Biochemistry, Uppsala. Reference values for FSH and LH in serum of healthy men and women have been reported previously (16).

RESULTS

Gel chromatographic studies of FSH in human serum

The protein distribution and the FSH and LH concentrations in the fractions eluted from gel chromatography of serum specimens from 8 individuals and one serum specimen to which pituitary FSH was added were determined. The K_{av}-values for albumin, FSH and LH were about 0.45, 0.48 and 0.54, respectively. The concentration of FSH and LH in the serum specimens and the differences in the K_{av}-values between the hormone peaks and the albumin peak are given in Table 1. The values for these differences fell within a range of 0.028 - 0.040 for FSH and 0.070 - 0.092 for LH and there was no sex hormone related difference in the elution pattern of FSH or LH.

Table 1. Gel chromatography of FSH and LH in human sera on Sephadex G-200.

Serum specimen	Hormone concentration		Difference in K_{av}-value	
Sex Years of age	FSH µg/l	LH µg/l	FSH-albumin	LH-albumin
Female				
4	0.16	-	0.039	-
28 follicular phase	1.05	0.71	0.036	0.092
32 after LRH	12.5	16.0	0.039	0.077
59	5.4	1.36	0.031	0.070
61	7.8	1.78	0.030	0.080
Male				
23	1.13	0.76	0.035	0.085
23 + pituitary FSH	6.0	-	0.040	-
26	0.33	0.30	0.028	0.080
66	1.67	0.84	0.038	0.076

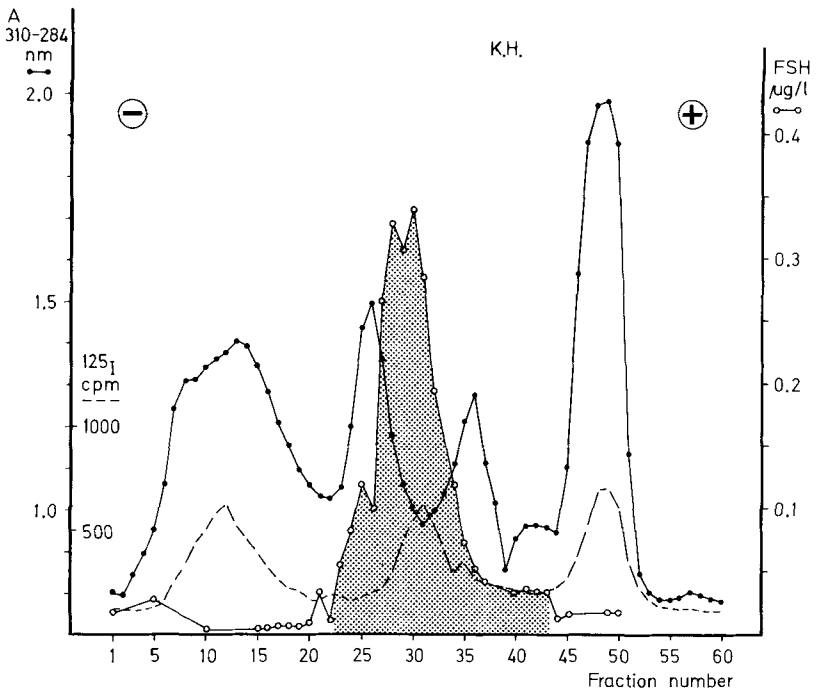

Fig. 1. Agarose suspension electrophoresis of 2 ml of serum from day 14/28 of a regularly menstruating 28 year old woman. The hatched line indicates the radioactivity from trace amounts of labelled LH (left), FSH (middle) and albumin (right) added to serum. The FSH activity in the serum is indicated by the dotted area.

Electrophoretic studies of FSH in human serum

The distributions of protein and FSH activity after electrophoresis in agarose suspension (column B) of 2 ml of a serum specimen taken on day 14/28 from a regularly menstruating 28 year old woman is shown in Fig. 1. The distribution of radioactivity is also indicated in the figure and labelled LH is eluted with a peak in fraction 12, FSH in fraction 31 and albumin in fraction 49. The position of the starting zone was fraction 7. The migration rate of the labelled FSH in relation to albumin was 56.24 ± 0.82 (SD) per cent for column A (n = 29) and 56.26 ± 0.29 per cent for column B (n = 10). The reproducibility expressed as the coefficient of variation of the migration rate of labelled FSH was 1.4 per cent for column A and 0.5 per cent for column B.

The electrophoretic patterns of FSH in serum from two young women, two post-menopausal women, and two men are shown in Fig. 2. The position of the labelled FSH is indicated by a vertical hatched line. FSH in serum from the two men and the two post-menopausal women was more negatively charged than the FSH in serum of the two young women. To study a possible change in the electrophoretic mobility of FSH in serum stored at -18°C for a long time a serum specimen from a

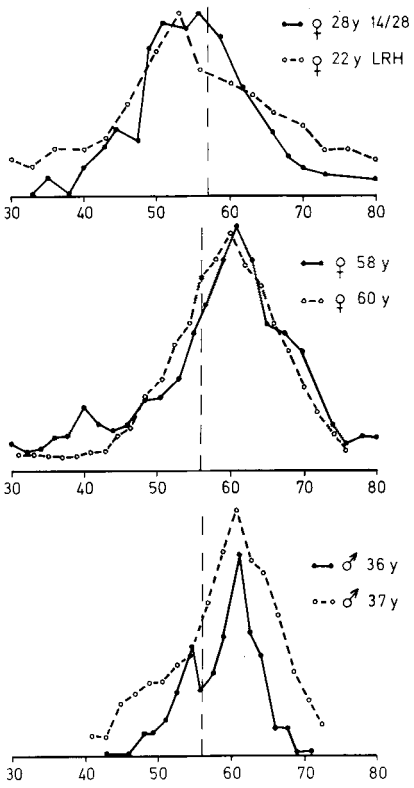

Fig. 2. Elution pattern of FSH from electrophoresis in agarose of serum from 2 young women, 2 post-menopausal women and 2 men. The hatched line indicates position of labelled FSH. Migration rate expressed in relation to albumin (=100). Anode to the right.

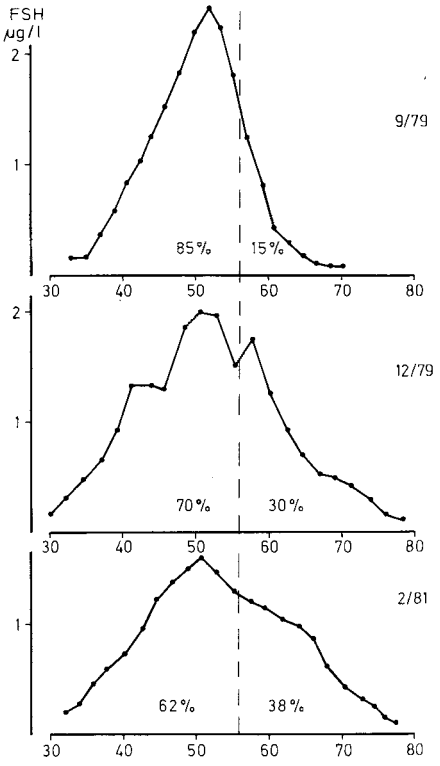

Fig. 3. Electrophoretic pattern of FSH in a serum specimen from a woman with an FSH producing pituitary adenoma. The serum was stored at -18°C and analysed after 6, 9 and 23 months. Migration rate expressed in relation to albumin (=100). Anode to the right.

79 year old woman with a pituitary adenoma (14, 15) was analysed by agarose suspension electrophoresis after 6, 9 and 23 months. The results are shown in Fig. 3. The main peak of FSH remained less acidic than the labelled FSH, but there was a gradual change in the pattern to more acidic forms during the long storage of the serum.

The migration rate of the main peak of FSH in serum expressed as a percentage of that of albumin for 31 serum specimens from 25 individuals are summarized in Table 2. The FSH in serum of post-menopausal women was more acidic than that of women of fertile age (20 - 30 years). The FSH in serum of three men was also more acidic than that of the young women. The values for 'male' FSH, 60.3 - 62.5 per cent, were similar to those obtained for post-menopausal women, 58.0 - 62.4 per cent. The FSH in the serum of the patient with the FSH producing pituitary adenoma was less acidic than FSH in all the other 28 serum specimens

Table 2. Agarose suspension electrophoresis of FSH in human serum.

Subjects Age in years	Number Individuals	Specimens	Migration rate, in per cent of that of albumin Mean	Range
Women, 20 - 30	5	6[1]	52.5	52.0 - 53.0
Women, 50 - 79	14	15[2]	59.9	58.0 - 62.4
Men, 36 - 67	3	3[2]	61.2	60.3 - 62.5
Woman with an FSH producing pituitary adenoma 79	1	3	50.8	49.5 - 51.7
Woman with premature menopause 25	1	2	60.6	60.4 - 60.7
Woman with primary amenorrhoea 20	1	2[2]	60.1	60.0 - 60.2

[1] Four specimens after LRH included. [2] One specimen after LRH included.

investigated. The values for FSH in serum of a woman (25 years) with a premature menopause and of a woman (20 years) with a primary amenorrhoea (delayed puberty?) were both near the mean value of FSH of post-menopausal women.

In two cases, serum specimens were taken before and after LRH injection. The migration rates of FSH were similar before and after LRH (59 versus 60 and 60 versus 60 per cent). Seven values obtained after LRH were included in Table 2. The concentration of FSH in the sera investigated, excluding those taken after LRH, was normal for sex and age in all cases with exception for the woman with the premature menopause and the woman with the FSH producing pituitary adenoma. The relationship between the position of FSH by electrophoresis and the concentration of FSH in serum is shown in Fig. 4. There was no significant ($p>0.05$) correlation between the concentration of FSH and the electrophoretic migration rate of FSH for the sera of the 14 post-menopausal women. The other groups were too small to permit any statistical comparisons of the two variables.

DISCUSSION

It was by use of gel chromatography that a sex hormone dependent variation in apparent molecular size of pituitary FSH was demonstrated for the rhesus monkey and the rat (3, 7, 8, 9). The results of the present study show that the degree of retardation by gel chromatography on Sephadex G-200 of FSH in human serum could be determined for both men and women and for a 4 year old girl. A significant sex hormone related difference in apparent molecular size like that found in the rhesus monkey and in the rat for pituitary FSH could not be

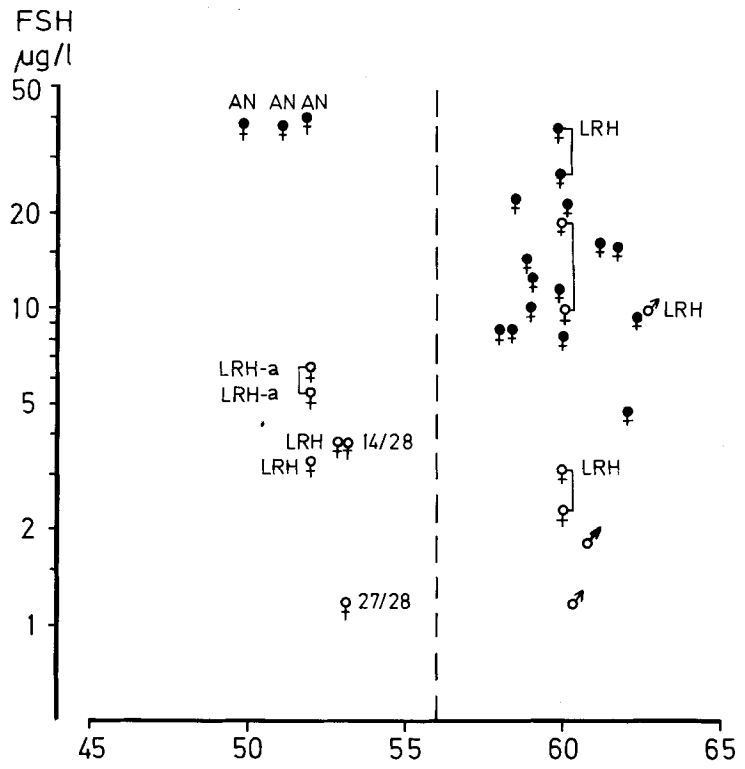

Fig. 4. Relationship between electrophoretic migration rate and concentration of FSH in 31 serum specimens from 25 individuals. Open circle on female symbol indicates woman 20 - 30 years of age and filled circle woman 50 - 79 years of age. AN = patient with pituitary adenoma. LRH-a = LRH agonist. 14/28 and 27/28 indicate day of cycle. Symbols for young women at position 60 represent one patient with primary amenorrhoea (lower FSH level) and one with premature menopause (higher FSH level). Migration rate expressed in relation to that of albumin (=100). Vertical hatched line indicates position of labelled FSH.

demonstrated for FSH in human serum. This does not exclude that small differences in molecular size exist between 'male' and 'female' FSH in man. The FSH in the different sera was eluted within a very narrow range in relation to albumin which may be due to the gel or the buffer used in the present investigation.

The agarose suspension electrophoretic technique permitted analysis of a large amount of proteins and up to 2 or 4 ml of serum could be analysed. With this technique a difference in charge of female FSH in human serum versus male and post-menopausal female FSH could be demonstrated. Particular advantages with this technique are that after electrophoresis the suspension can be pumped out from the electrophoretic tube and collected in a sample collector with negligible distortion of the zones, and that after centrifugation of the agarose the hormone assays can be made on the supernatant without any need of pH adjustment or other treatment. The electrophoretic migration velocities of proteins in these

agarose suspensions have been shown to be similar to those in free solution (4). The solid phase radioimmunoassay permits determinations in large volumes and a very high sensitivity was obtained by a long preincubation before addition of labelled hormone.

It was not possible to determine the pI-values of the different components and the electrophoretic behaviour was therefore expressed as migration rate in relation to that of albumin. Labelled FSH was found to be a suitable control marker as it had a migration rate between that of FSH in sera of young women and that of post-menopausal women or men. The labelled FSH was included in each run and used for calculation of the reproducibility of the electrophoresis. The method was extremely reproducible with values for coefficient of variation for the labelled FSH of 0.5 and 1.4 per cent for two of the columns used.

Similar electrophoretic studies on possible sex hormone related pleomorphism of FSH of human or animal sources seem not to have been made previously. There may be considerable species differences as indicated by the results obtained in the rhesus monkey and the rat by the use of gel chromatography. The pleomorphism of FSH which was observed for FSH in serum may be due to differences in the amino acid or carbohydrate composition. Small differences in the number of amide groups or of sialic acid residues may give changes in charge which can explain the different migration rates by electrophoresis.

The results strongly indicate that there is in man a sex hormone related pleomorphism of FSH with less acidic forms of FSH circulating in the serum of normal women of fertile age than in serum of men and post-menopausal women. The biological significance of this pleomorfism is not known. The different forms of FSH may have different rates of disappearance from the circulation as was shown for pituitary FSH in the rhesus monkey and the rat. Qualitative analyses of FSH in serum by electrophoresis may become an important complement to the quantitative determinations of the FSH concentration for the clinical diagnosis of reproductive disorders and of FSH producing tumours.

ACKNOWLEDGEMENTS

I am grateful to Mr Christer Bengtsson and Miss Karin Eriksson for skilful technical assistance, and to Dr. P. Roos, Uppsala for purified FSH and LH preparations. This work was supported by the Swedish Medical Research Council (grant 637).

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Received May 15, 1981

Address for reprints:

Leif Wide, M.D.
Department of Clinical Chemistry
University Hospital
S-750 14 Uppsala 14
Sweden