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Research Article

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Metabolic Clearance and Production Rates of Human Luteinizing Hormone in Pre- and Postmenopausal Women

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ABSTRACT Metabolic clearance rates and production rates of human luteinizing hormone (HLH) were determined in pre- and postmenopausal women by the constant infusion technique. Highly purified HLH-^{131}I was infused into the fasting subjects at a constant rate. Serial plasma samples were obtained and the radioactive hormone was precipitated by a double antibody technique. Plasma HLH-^{131}I levels reached equilibrium by 4 hr after the infusion started. Metabolic clearance rates were: 24.4 ± 1.8 (mean ± SE) ml/min in five normal premenopausal women; 23.3 ± 1.1 ml/min in five normal women taking norethinodrel and mestranol; and 25.6 ± 4.1 ml/min in four postmenopausal women. Endogenous plasma HLH levels measured in the same subjects by radioimmunoassay immediately before infusion were 32.0 ± 9.6 mU/ml in the normal women, 16.8 ± 3.2 mU/ml in the women on oral contraceptives, and 99.2 ± 23.2 mU/ml in the postmenopausal women. The corresponding HLH production rates were: 734 ± 170 mU/min in the normal women; 387 ± 86 mU/min in the women on norethinodrel and mestranol; and 2400 ± 410 mU/min in the postmenopausal women. The metabolic clearance rate did not change after ovariectomy in one women, but the production rate rose from 583 to 1420 mU/min. Based on previously reported bioassay values for pituitary content and urinary excretion of HLH, the estimated turnover of HLH in the pituitary is about once per day and less than 5% of the total HLH produced appears in the urine in a biologically active form.

INTRODUCTION

Most of the information available on human gonadotropin physiology has been derived from bioassay data on extracts of large volumes of plasma or urine. The quantity of these fluids required for bioassay has prevented sequential studies at short intervals in a single patient. The recent preparation of highly purified human luteinizing hormone (HLH) (1) and the development of a sensitive radioimmunoassay (2) with which HLH can be easily quantified in small samples of plasma or serum have now made it possible to estimate the pituitary secretion or production rate (PR) of HLH.

The purpose of the present study was to estimate the PR of HLH using the plasma clearance of HLH-^{131}I and the endogenous plasma HLH level. Initial studies on plasma HLH-^{131}I levels after a single injection showed a rapid multiexponential disappearance curve, never quite reaching a clearly linear slope during the time HLH-^{131}I blood levels could be reliably determined. Therefore, we have utilized the constant infusion (to equilibrium) method of Tait (3, 4) to estimate the metabolic clearance rate (MCR) or HLH-^{131}I from plasma.

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METHODS

Preparation of HLH-^{131}I. Highly purified HLH (1) was labeled to specific activities of 50–150 $\mu c/\mu g$ with ^{131}I by a modification of the method of Greenwood et al. (5). Exposure of the hormone to Chloramine T was limited to 15–20 sec before addition of sodium metabisulfite to avoid unnecessary damage to the hormone. HLH-^{131}I at these specific activities contains about one ^{131}I atom per five molecules of HLH. The relative abundance of ^{131}I per total iodide in so-called "carrier-free ^{131}I" [1] has been found to range from 5 to 44% (6). Therefore, the ratio of total iodide atoms to HLH molecules was about 1 : 1. Immediately after iodination the mixture was passed through a Sephadex G-75 column. The fraction selected for studies showed essentially no damaged HLH-^{131}I or free ^{131}I by hydrodynamic flow chromatoelectrophoresis on Whatman 3 MM paper (Fig. 1). The HLH-^{131}I was sterilized by Millipore filtration, cultured, and pyrogen tested. The interval between iodination and testing was 2–5 days.

Measurement of HLH-^{131}I. HLH-^{131}I was precipitated from plasma or solution by a double antibody system (7). Aliquots of 1 ml were treated with excess rabbit anti-HLH at 4°C for 24 hr. A sheep anti-rabbit globulin was added in excess, and the mixture was incubated for an additional 24 hr. The samples were centrifuged, and the supernatant was removed by suction. Total and precipitated radioactivity were determined on duplicate aliquots in a well-type scintillation counter. Greater than 97% of the fraction used for studies was precipitable in a double antibody system with excess antibody on the day of iodination.

Measurement of endogenous HLH. Plasma HLH was determined by radioimmunoassay as previously described (2).

Plasma HLH disappearance studies. Preliminary studies were performed to determine the feasibility of using the disappearance curve of HLH-^{131}I from plasma after a single injection to determine the MCR and PR of HLH. The disappearance of total and antibody precipitable radioactivity from plasma after a single bolus injection of HLH-^{131}I was investigated in four patients: three with chromophobe adenomas (a 45 yr old woman, a 62 yr old woman, and a 44 yr old man) and a 40 yr old woman with carcinoma of the breast. All patients were given five drops of saturated potassium iodide solution every 6 hr for 24 hr before study to inhibit ^{131}I uptake by the thyroid. 12–25 μc of HLH-^{131}I which contained the equivalent of 1.6–3.2 U [2] were injected intravenously in 0.5–1.0 ml of saline. Serial 10-ml heparinized venous blood samples were obtained over a 24 hr period. The patients were kept at bed rest and received a liquid diet during the study. Urine was collected periodically for 24 hr.

Because the HLH-^{131}I disappearance curve after the single injection appeared multiexponential, the MCR was calculated according to the formula for any system of pools (3, 8):

$$MCR = \frac{R}{\int_0^\infty x' \cdot dt}$$

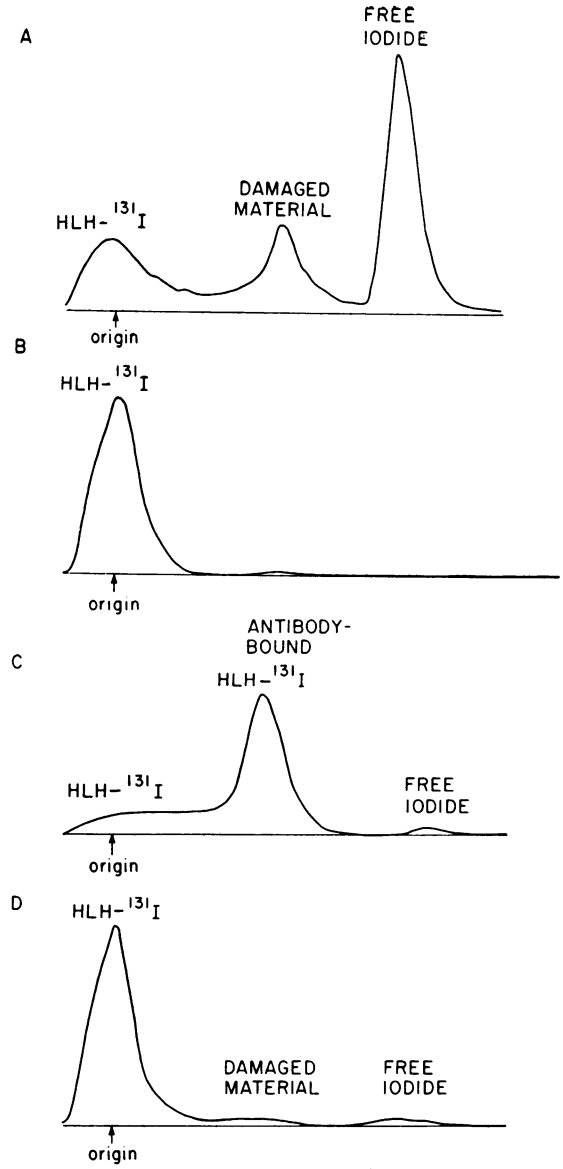

FIGURE 1 Scans of paper chromatoelectrophoretograms of HLH-^{131}I after iodination. Origin is near cathode, and anode is to the right. A is the total iodination mixture showing HLH-^{131}I, "damaged HLH-^{131}I," and free ^{131}I. B is the fraction used for clearance studies immediately after passage through a G-75 Sephadex column. C is the same fraction as B after incubation with excess anti-HLH for 1 hr, showing that the material adhering to the origin is immunoreactive. D is the B fraction as prepared for infusion.

[1] Obtained from Iso-Serve, Cambridge, Mass.

[2] In terms of International Reference Preparation of Human Menopausal Gonadotropin No. 2.

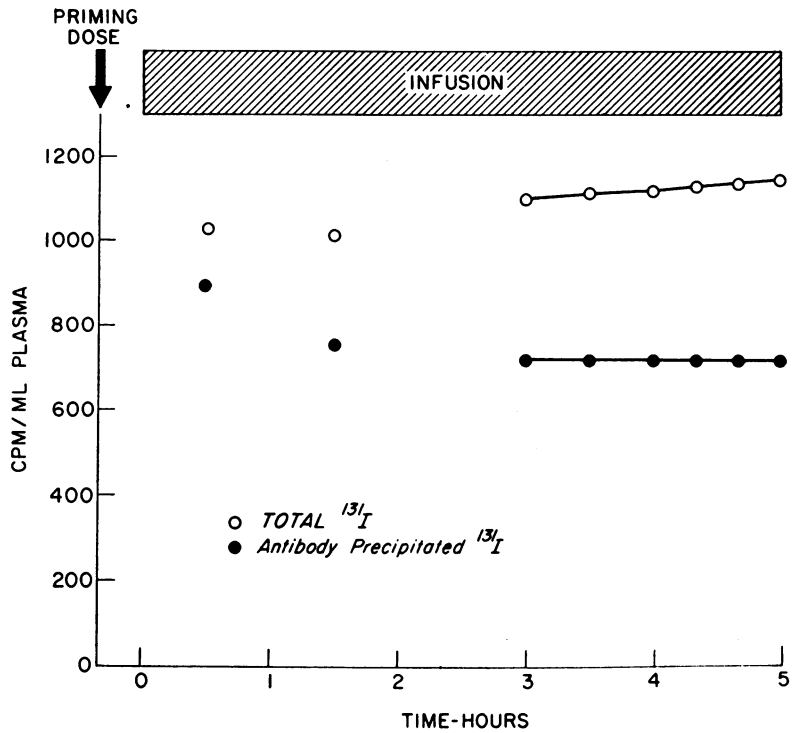

FIGURE 2 Schematic representation of infusion technique. A priming dose of HLH-^{131}I was given 20 min before starting the infusion. Blood was drawn at 20–30-min intervals over the last 2 hr of the infusion. Total and antibody precipitable ^{131}I were determined on duplicate aliquots of plasma.

where R is the total HLH-^{131}I injected as a single dose, and x' is the plasma concentration of antibody precipitable radioactivity. The integral $\int_0^\infty x' \cdot dt$ was determined by plotting the measured plasma HLH-^{131}I concentration against time after administration and by numerically measuring the area under the disappearance curve (8). The production rate (PR) of HLH was then calculated as

$$PR = MCR \cdot i$$

where i is the concentration of endogenous unlabeled HLH determined by radioimmunoassay (3).

The disappearance of *endogenous* unlabeled HLH from plasma was also determined on one patient at the time of hypophysectomy for palliation of carcinoma of the breast. This patient had previously had a bilateral ovariectomy and the plasma HLH level was elevated. Plasma HLH was measured in serial blood samples taken during the surgery. Because plasma HLH levels did not fall to undetectable levels, the residual HLH concentration after hypophysectomy was subtracted from each sample to show the disappearance curve. The failure of the plasma HLH to fall to zero was the result of incomplete hypophysectomy. This was confirmed by the finding of biologically active gonadotropin in the urine after surgery.

Continuous HLH-^{131}I infusion studies. Three groups of subjects were used for these studies: (*a*) five normal premenopausal women studied at random times during the menstrual cycle; (*b*) five normal premenopausal women taking norethinodrel and mestranol (5 mg daily on day 1 through 20 of cycle) for contraceptive purposes; and (*c*) four postmenopausal women. The latter group included two normal women and two women with carcinoma of the breast with no evidence of extensive hepatic or other metastases at the time of study. The subjects were again prepared with five drops of saturated potassium iodide every 6 hr for 24 hr before study. The infusion tests were initiated between 6:00 and 10:00 a.m. with the subjects fasting and at bed rest. A 10 ml heparinized sample of blood was drawn immediately before the test for endogenous HLH determination. A total dose of 5–25 μc of HLH-^{131}I containing 0.8–3.2 U of HLH was given to each subject. One-fifth of the total dose was given directly intravenously as a priming dose 20 min before starting the infusion. The remainder of the HLH-^{131}I was infused in a 1% albumin solution in saline at a constant rate of 1.25–1.30 ml/min over a 3–6 hr period.

The HLH-^{131}I was measured in the infusion solution by antibody precipitation. Only the immunoreactive radioactivity (80–95%) was used in calculating the rate of infusion. The concentration of antibody precipitable

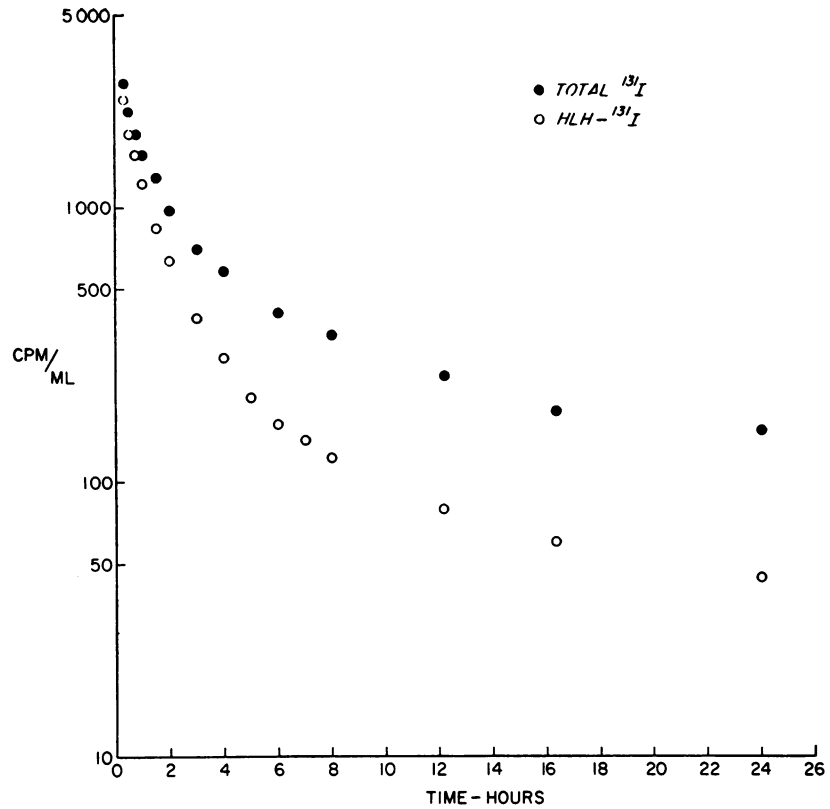

FIGURE 3 Typical disappearance curve of HLH-^{131}I from plasma after a single injection in a 40 yr old woman. Note that there is no linear segment of the curve.

HLH-^{131}I in the infusion solution did not change throughout the test.

10-ml heparinized blood samples were drawn every 20–30 min for the last 2 hr of the infusion (Fig. 2). Four or more samples were used to calculate the mean plasma HLH-^{131}I at equilibrium. As a control for nonspecific degradation of HLH-^{131}I by plasma, the HLH-^{131}I was placed in plasma and incubated at 37°C. The loss of immunoreactivity was less than 2% from 0 to 6 hr and was only 5% at 24 hr.

The plasma MCR defined as the volume of blood cleared completely and irreversibly of HLH-^{131}I in unit time was calculated after the method of Tait (3, 4) according to the following formula:

$$\text{MCR} = \frac{r}{x'c}$$

where r is the rate of infusion of HLH-^{131}I in cpm per minute and $x'c$ is the plasma HLH-^{131}I level after equilibrium has been reached in cpm per ml. The production rate (PR) of HLH or secretion into the plasma is again calculated as

$$\text{PR} = \text{MCR} \cdot i.$$

RESULTS

Plasma HLH disappearance studies. The disappearance curve of HLH-^{131}I from plasma after a single injection showed a multiexponential type of fall with an initial half-time between 30 and 60 min (Figs. 3 and 4). The divergent total plasma ^{131}I curve was interpreted as showing the return of degradation products of HLH-^{131}I back into the plasma. The antibody precipitable radioactivity fell from a mean of 0.027% of the injected dose per ml of plasma at 20 min to 0.00058% at 24 hr. The per cent of the total plasma radioactivity which was antibody preciptable fell from a mean of 87% at 20 min to 31% at 24 hr. Urinary excretion of the total injected ^{131}I ranged from 62 to 99.5% over the first 24 hr with fairly constant excretion of 10–15% per hour for the first 5 hr. Antibody precipitable radioactivity in the urine was always less than 0.1% of the total.

The type of disappearance curve of HLH-^{131}I from plasma suggested that the HLH-^{131}I was dis-

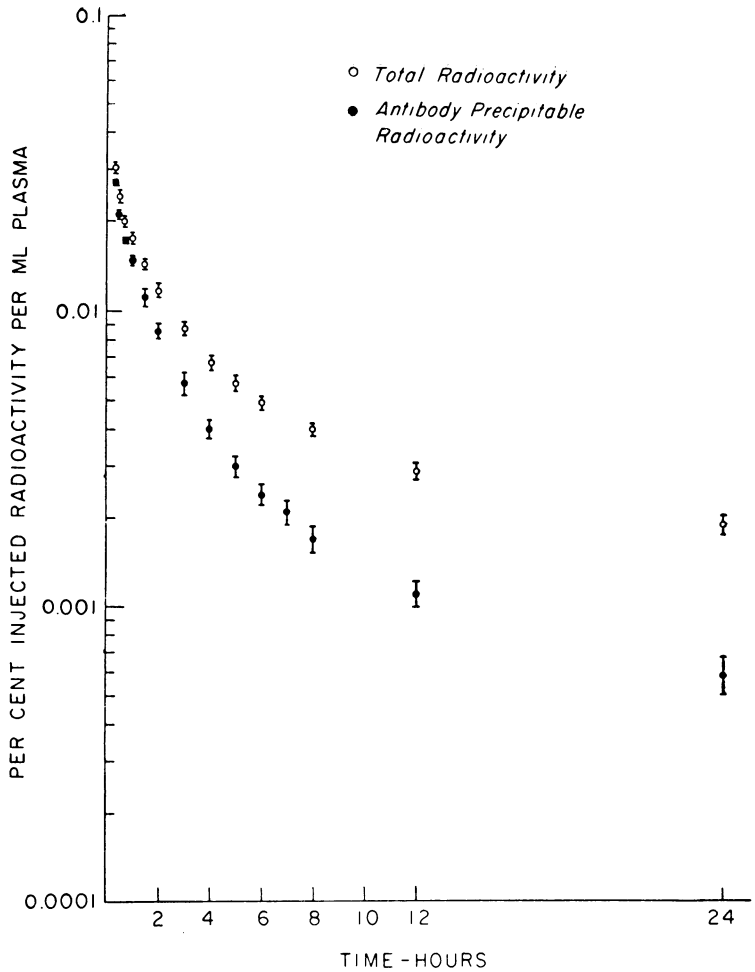

FIGURE 4 The disappearance curves of HLH-^{131}I from plasma in four patients were similar. Each point is the mean ± SE of the individual determinations in the four patients tested.

tributed in more than three mathematical compartments. The semilogarithmic plot of the curve showed no definitely linear segment during the first 24 hr although most of the labeled hormone had been degraded during this time as shown by the total plasma ^{131}I and urinary ^{131}I excretion. The MCR's and PR's of HLH calculated from the single injection studies are shown in Table I. Although the MCR and PR could be calculated by the single injection technique, the disappearance curve of HLH-^{131}I was complex, and the area under the curve had to be mechanically or numerically integrated. Therefore, the constant infusion technique was used for subsequent studies.

TABLE I

Metabolic Clearance Rates and Production Rates of HLH Estimated by the Single Injection Technique

Age	Sex	Diagnosis	MCR	Plasma HLH	PR
			ml/ min	*mU/ ml*	*mU/ min*
45	F	Chromophobe adenoma	19.6	<4.5	—
62	F	Chromophobe adenoma	19.8	42.4	839
40	F	Carcinoma of breast*	19.6	28.0	549
44	M	Chromophobe adenoma	20.3	7.4	150

* Patient previously ovariectomized.

The disappearance curve of endogenous HLH from the plasma of the patient during hypophysectomy was not entirely satisfactory because blood was given to the patient during the procedure. However, before blood administration, the HLH

fell with a half-time of 30–60 min. Fig. 5 shows a comparison of the disappearance curves of HLH-^{131}I after a single injection and of endogenous HLH at hypophysectomy.

Continuous HLH-^{131}I infusion studies. Plasma HLH-^{131}I levels appeared constant by hr 3 or 4 of the infusion in 13 of 14 subjects. The mean and SD of the last four or more values used in calculating the MCR are shown in Table II. In only one subject (subject 3) was there greater than 5% variation of any sample from the mean during the last hour of infusion. To further examine for evidence that equilibrium had been reached, the plasma HLH-^{131}I levels were expressed as a per cent of the final concentration and inspected for the presence of an upward or downward trend. Only subject 3 continued to show a slight upward trend of plasma HLH-^{131}I levels over the last hour of infusion. However, her values appeared sufficiently near equilibrium to include with the others (see Table II). The MCR's were: 24.4 ± 1.8 (mean ± SD) ml/min in the five normal premenopausal women; 23.3 ± 1.1 ml/min in five

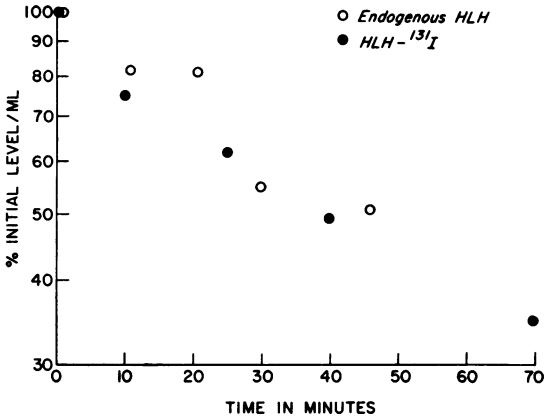

FIGURE 5 A comparison of the disappearance curves of HLH-^{131}I after a single injection and of endogenous HLH at hypophysectomy. The 0 time on the HLH-^{131}I curve actually represents samples at 20 min after injection, but the curve was arbitrarily shifted to the left to allow for rapid mixing.

TABLE II

Results of Continuous Infusion Studies of HLH-^{131}I in Pre- and Postmenopausal Women

Patient	Age	Weight	Height	Body surface area	Day of menstrual cycle	Precipitable cpm infused	Blood level at equilibrium	MCR	Plasma LH	PR
	yr	*Kg*	*cm*	*m²*	*day*	*cpm/min*	*cpm/ml* ± SD	*ml/min*	*mU/ml**	*mU/min**
Premenopausal women										
1	29	64	155	1.61	2	20,027	723 ± 5	28.0	13.6	381
2	24	58	173	1.68	19	15,306	603 ± 7	25.4	28.0	711
3	26	58	172	1.67	2	19,937	889 ± 52	22.4	16.8	376
4	20	47	154	1.41	20	14,074	763 ± 22	18.4	68.8	1266
5	25	55	157	1.53	24	15,306	550 ± 10	27.8	33.6	935
Mean ± SE								24.4 ± 1.8	32.0 ± 9.6	734 ± 170
Premenopausal women on norethinodrel and mestranol										
6	22	59	169	1.67	9	30,600	1362 ± 12	22.5	9.6	216
7	23	58	161	1.59	11	11,539	607 ± 4	19.0	22.4	426
8	22	61	168	1.69	22	9,740	410 ± 14	23.8	12.8	305
9	25	63	171	1.73	10	15,153	552 ± 8	27.5	25.6	704
10	22	52	160	1.51	21	5,953	250 ± 17	23.8	12.0	286
Mean ± SE								23.3 ± 1.1	16.5 ± 3.2	387 ± 86
Postmenopausal women										
11	45	49	153	1.43	—	18,988	882 ± 33	21.5	98.4	2120
12	39	65	156	1.63	—	20,924	1022 ± 29	20.5	164.0	3360
13	49	83	154	1.81	—	13,637	601 ± 15	22.7	62.4	1420
14	39	64	175	1.77	—	10,596	281 ± 10	37.7	72.0	2710
Mean ± SE								25.6 ± 4.1	99.2 ± 23.2	2400 ± 410

* In terms of the Second International Reference Preparation of Human Menopausal Gonadotropin.

normal women taking oral contraceptives; and 25.6 ± 4.1 ml/min in the four postmenopausal women. There were no significant differences among groups ($P > 0.05$). The higher endogenous plasma HLH levels in the premenopausal women on no medications were due in part to the various times in the menstrual cycle when the studies were performed. Subject 4 was apparently having an ovulatory HLH surge at the time of study. The PR's were: 734 ± 170 mU/min in the normal premenopausal women; 387 ± 86 mU/min in the five women on norethinodrel and mestranol; and 2400 ± 420 mU/min in the four postmenopausal women. The patient who underwent ovariectomy was 49 yr old but was having regular menses before surgery. The preoperative MCR was 24.3 ml/min with a plasma HLH of 24.0 mU/ml and a PR of 583 mU/min. The postoperative MCR was 22.7 ml/min with a plasma HLH of 62.4 mU/ml and a PR of 1420 mU/min.

DISCUSSION

There are no previously published estimates of the secretion rate of HLH. In general, methodology has not been available to permit such studies. Highly purified HLH labeled with radioiodine can be used in tracer amounts for metabolic studies in doses that are not supraphysiologic. However, before an iodinated protein can be used as metabolic tracer in vivo, it must be shown to be indistinguishable from the unlabeled protein in that particular system under study.

The assumption that HLH-^{131}I was cleared from the plasma at the same rate as endogenous HLH is critical to the validity of the present study. This assumption seems justified on the basis of studies on the disappearance curve of HLH in the serum of monkeys.[3] Because highly purified HLH was not available in sufficient quantity for extensive clearance studies, a preparation of human menopausal gonadotropin was injected simultaneously with the purified HLH-^{131}I into five Macaca mulatta monkeys. The disappearance of antibody precipitable HLH-^{131}I was compared with the disappearance of HLH determined by radioimmunoassay. The disappearance curve in plasma was indistinguishable for the HLH-^{131}I and unlabeled HLH. The additional finding of a similar disappearance curve of HLH-^{131}I and endogenous HLH at the time of hypophysectomy in one patient (Fig. 5) suggests that the lightly iodinated HLH is cleared from the plasma at the same rate as unlabeled HLH. Unfortunately, a method other than hypophysectomy for acutely suppressing plasma HLH levels is not yet available to facilitate endogenous plasma HLH half-time determinations.

Another assumption necessary to validate production rate studies is that a steady state is maintained in regard to secretion and degradation of the endogenous hormone during the test period. The degradation of HLH-^{131}I must reach a steady rate during the constant infusion studies. Studies of diurnal HLH variation have shown no significant change over the course of 24 hr in women except during the HLH surge at the time of ovulation.[4] Therefore, it appears that steady rates of HLH production and degradation were probably maintained throughout the period of these studies. The possible exception is subject 4 who was apparently having an ovulatory HLH elevation.

The finding that plasma HLH-^{131}I in humans did not have a linear disappearance rate on a semilogarithmic plot after a single injection is of interest. Disappearance curves in the literature for ^{131}I-labeled insulin (9), glucagon (10), growth hormone (11), and thyrotropin (12) after a single injection appear to show an initial linear segment after a mixing period followed by a slower decline. These disappearance curves are in good agreement with the disappearance of appropriate unlabeled hormones in these studies. If a single compartment of distribution is assumed, fractional turnover rates can easily be calculated from these data. In the case of HLH, however, there appears to be a definite multiexponential disappearance curve. Although the disappearance curves of HLH-^{131}I and unlabeled HLH again appear to be in good agreement in the one patient at hypophysectomy and in primates, the initial disappearance is not linear and the single compartment assumption cannot be made. A meaningful MCR in a multicompartmental system should be a function of the total integrated area under the disappearance

[3] Kohler, P. O., G. T. Ross, W. W. Tullner, J. M. Phang, and W. D. Odell. In preparation.

[4] Ross, G. T., W. D. Odell, and P. L. Rayford. Unpublished observations.

curve. Highly complex curves are mathematically difficult to resolve and it becomes necessary to integrate mechanically or numerically the area under the curve. The continuous infusion method (3, 4) provides a simple means of integrating the area under the disappearance curve and is a valid technique for determining the clearance rate in both single and multicompartmental systems.

Another possible interpretation of the disappearance curves after the single injections is that some damaged HLH-^{131}I was present. Iodination of smaller peptides such as alpha MSH (13) and arginine vasopressin (14) can result in a slowing of the disappearance curve. However, proteins smaller than HLH such as insulin (9), glucagon (10), and growth hormone (11, 15), or equivalent in size and composition, such as thyrotropin (12), can be lightly iodinated without significantly changing the disappearance curve. Furthermore, the fraction used for the present studies showed less than 1% damaged HLH-^{131}I by chromatoelectrophoresis and only immunoreactive HLH-^{131}I was used in determining the infusion rates. It is very possible that a small fraction of damaged HLH-^{131}I might result in the slow disappearance indicated by the late portion of the plasma HLH-^{131}I curve after the single injection, at a time when most of the labeled hormone had already been degraded. However, the error in clearance determinations of such a small fraction would be almost negligible over the time period of the constant infusion method. We also have found that human menopausal gonadotropin with almost no FSH activity can be iodinated with ^{127}I using Chloramine T, total iodide, and sodium metabisulfite in ratios proportional to those used for the present study with minimal or no loss of biologic LH potency as determined by the rat ventral prostate assay.[5]

Tait (3, 4) has described the rationale for using the constant infusion-to-equilibrium method for determining the metabolic clearance rates of steroids. This method has not previously been applied to polypeptide hormones, but is equally applicable when it can be shown that the labeled hormone behaves in an identical manner to the unlabeled hormone in the system under study. The constant infusion approach can be regarded as imitating the HLH secretion of the pituitary by using HLH-^{131}I. If the labeled hormone is cleared from the plasma at the same rate as the endogenous hormone, the ratio of the rate of HLH-^{131}I infusion to the final plasma HLH-^{131}I level at equilibrium will be equivalent to the ratio of the secretion of endogenous HLH to the endogenous plasma HLH level. The similar MCR values found by the single injection and constant infusion techniques suggest that either may be used for MCR and PR determinations. However, the constant infusion technique provides some advantages over the single injection method. The area under the infusion curve is automatically integrated, and factors which influence the MCR can be followed in the same subject (3). The shorter time period for infusion of HLH-^{131}I reduces nonspecific damage to the iodinated hormone which could result in spuriously low MCR and PR values.

The present data from the constant infusion studies clearly show that there is little difference in the MCR of HLH among three groups of subjects with rather markedly differing plasma HLH levels. The studies before and after ovariectomy in one patient also showed no change in MCR while the plasma HLH more than doubled. These findings indicate that the fluctuations noted in plasma HLH levels are the result of changes in the rate of HLH secretion rather than the rate of HLH clearance. Although subject 4 who had the lowest body surface area also had the lowest MCR, generally the MCR did not correlate ($P > 0.05$) with height, weight, or body surface area.

Ryan (16) has found that the HLH content in the pituitaries of five healthy young premenopausal women dying suddenly ranges from 0.162 to 2.209 mg of NIH-LH-S1 per pituitary (mean = 1.445) by the ovarian ascorbic acid depletion assay (17). The HLH content in the pituitaries of four postmenopausal women was 1.191–5.462 mg of NIH-LH-S1 per pituitary (mean = 3.453). Rosemberg and Lewis (18) have found 1 mg of NIH-LH-S1 to be equivalent to 500 U of the 2nd International Reference Preparation of Human Menopausal Gonadotropin by the ovarian ascorbic acid depletion assay. The total pituitary HLH content, therefore, averaged about 725 U in the premenopausal and 1725 U in the postmenopausal women. If the HLH PR's were to be maintained at a relatively

[5] Kohler, P. O., G. T. Ross, W. W. Tullner, J. M. Phang, and W. D. Odell. In preparation.

stable level for 24 hr as suggested from the lack of significant diurnal variation except during the ovulatory surge, the premenopausal women would have a secretion of about 500–1100 U/day, and the postmenopausal women would have a secretion of about 3500 U/day. These values would represent a one- to twofold turnover of total pituitary HLH content daily in both groups. This estimated daily pituitary turnover of HLH is similar to the value reported for human thyrotropin (12) and suggests that the secretion of both hormones is an extremely active process with a rather rapid turnover rate in the pituitary.

The amount of HLH-^{131}I that could be precipitated from the urine in the present study was less than 0.1%. This value would suggest a low renal clearance of undegraded HLH. However, although we have shown that the clearance of HLH-^{131}I from plasma is similar to unlabeled HLH, we have no evidence that the renal clearance of HLH-^{131}I is the same as unlabeled HLH. Keller (19) has found that the renal clearance of HLH by bioassay is between 0.03 and 0.26 ml/min in postmenopausal women. When these values are compared to our total MCR's of 18.4–37.7 ml/min from the present study, it appears that less than 5% of the total HLH production actually appears in the urine in the biologically active form. Becker and Albert (20) have reported that the normal values for urinary excretion of HLH by the rat ventral prostate assay (21) are from 0.21 to 0.43 mg equivalents of NIH-LH-S1 per 24 hr in premenopausal women and 2.1 mg equivalents of NIH-LH-S1 per 24 hr in postmenopausal women. After conversion to International Units, according to Rosemberg and Lewis (18) ($\times$ 62.5 for rat ventral prostate assay), the values for premenopausal women are 13.1–25 U/24 hr in premenopausal and 131.3 U/24 hr in postmenopausal women. Again, it would appear that less than 5% of the total daily HLH production can be measured in the urine by bioassay. There is no reason to believe that the renal clearance of biologically active HLH is important to hormone activity. It is of interest that urinary gonadotropin values which have been clinically invaluable in the past have probably been derived from a very small fraction of the total HLH production.

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