The Metabolism of Human Sex Hormone-Binding Globulin in the Rhesus Monkey (43502)

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Abstract. **The metabolism of human sex hormone-binding globulin (hSHBG) was studied in eight female rhesus monkeys (*Macaca mulatta*) after the pulse injection of [^{125}I]-hSHBG. hSHBG was iodinated with ^{125}I using a chloramine T technique, and the [^{125}I]-hSHBG was separated from other constituents by molecular sieve chromatography with a Sephadex G-25 column. The [^{125}I]-hSHBG was administered intravenously as a pulse in 2 ml of phosphate buffer, pH 7.4, to each of eight rhesus monkeys. Blood samples (2.0 ml) were obtained at 2, 4, 6, 8, 24, 30, 45, and 54 hr after the injection. The glycoproteins were precipitated with concanavalin A-Sepharose, and the radioactivity was measured.**

The concentration of radioactivity as fraction of dose/ml of serum was plotted on a semilog scale against time. The disappearance of radioactivity could be expressed best as the sum of two exponentials, with a mean ± SE $t_{½}$ of 2.5 ± 0.4 and 33.1 ± 3.7 hr, respectively. The initial volume of distribution was 461 ± 78 ml and the metabolic clearance rate was 559 ± 66 ml/day.

The very low clearance rate and prolonged $t_{½}$ are compatible with a relative stability in the circulating mass of SHBG. Rapid changes in concentration of SHBG could be due to changes in serum volume, reversible changes in tissue distribution of SHBG, or the secretion of variable forms of desialylated SHBG. [P.S.E.B.M. 1992, Vol 201]

Sex hormone-binding globulin (SHBG) has been the subject of recent investigations into its structure (1), species distribution (2), role in androgen and estrogen entry into tissues (3–5), and synthesis control (6). Although a role of metabolism as opposed to synthesis, to explain changes in concentration, has been suggested (7), there have been few studies on the actual metabolism of SHBG.

SHBG is highly glycosylated (1) and, like other glycoproteins, its rate of metabolism may accordingly be slow (8–11). Suzuki and Sinohara (12) reported on the rapid hepatic uptake in the rat of desialylated bovine SHBG as compared with native bovine SHBG, but there are few studies in higher species. There is a strong structural homology between human SHBG (hSHBG) and rhesus SHBG (rSHBG) (13), and Petra *et al.* (14) reported that hSHBG and rSHBG had similar effects on the metabolic clearance rate (MCR) of testosterone when infused into rhesus monkeys. Therefore, in order to study the metabolism of SHBG, we injected hSHBG into rhesus monkeys, and our findings provide the basis for this report.

Materials and Methods

The female rhesus monkeys (*Macaca mulatta*) were housed in individual cages and fed standard monkey pellets (Prolab primate 18; Agway Inc., Syracuse, NY). The monkeys had been ovariectomized previously and used in other studies (15, 16). At the time of the present studies, no monkey had received hormonal therapy or had been subjected to any surgery for at least 4 months. The [^{125}I]-hSHBG was administered intravenously to the monkeys under ketamine intramuscular anesthesia (7 mg/kg). Blood samples (2.0 ml) were drawn at 0, 2, 4, 6, 8, 24, 30, 45, and 54 hr. Venipuncture was performed with chair restraint or ketamine

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intramuscularly. All procedures were approved by the Institutional Animal Care and Use Committee.

In seven of the eight experiments, the hSHBG used was obtained from Chemicon Co. (Temecula, CA). This material was obtained in 10-μg amounts as needed. As noted below, the material ran as a single band on sodium dodecyl sulfate-polyacrylamide gels after iodination, but the exact state of its sialylation was not known. In one experiment, in monkey 35, the hSHBG used was a gift from Dr. Geoffrey Hammond (London Regional Cancer Centre, London, Ontario, Canada).

For each experiment, the hSHBG was iodinated using a modified version of the chloramine T procedure of Greenwood *et al.* (17). Two micrograms of hSHBG in 0.01 *M* phosphate-buffered saline (pH 7.6) were iodinated using 2 mCi of carrier-free ^{125}I (Amersham, Arlington Heights, IL) in a microfuge tube containing 125 μl of 0.5 *M* Na phosphate (pH 7.6) and 3.3 μg of chloramine T. After 20 sec, the reaction was stopped by the addition of 120 μg of sodium metabisulfite. Then 200 μl of 1% KI (w/v) in 16% sucrose (w/v) were added and the solution was mixed. The solution was applied to a Sephadex G-25 column (1 × 50 cm) equilibrated with 0.01 *M* phosphate-buffered saline containing 2% bovine serum albumin (pH 7.6). Eighty 1.0-ml fractions were collected by elution with 0.01 *M* phosphate-buffered saline. The radioactivity in a 10-μl aliquot of each tube was measured to determine the protein and iodine peaks. The contents of the tubes containing the protein peak were pooled for later administration, as described below. Analysis of ^{125}I-hSHBG by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, using a 4% polyacrylamide stacking gel and a 12% resolving gel, under reducing conditions revealed a single band at ≈47 kDa. It is probable that the additional band (18, 19) was not seen because either too little radioactivity was applied or the two bands were not resolved by the gel.

The iodination procedure was carried out in the afternoon 1 day before the injection. Prior to the injection, a 10-μl aliquot of the injection solution was removed and counted for its radioactivity. Then to this aliquot were added 1.5 ml of concanavalin A-Sepharose (Con A-Sepharose; Pharmacia, Uppsala, Sweden). The solution was mixed well three times and allowed to settle 20 min after each mixing. After the last mix and settling, the supernatant was carefully removed and the Con A-Sepharose was washed two times, as described above with buffer, and the buffer was removed and the radioactivity associated with the Con A-Sepharose was measured in a LKB gamma spectrometer (Scintiscan 1200; LKB, Gaithersburg, MD). The dose of radioactivity injected was determined from the measurements of radioactivity in the sample before and after Con A-Sepharose treatment. The radioactivity in the pre- and post-Con A-Sepharose samples differed by less than 20%, which indicates that little free iodine or desialylated [^{125}I]-SHBG was injected.

Each blood sample was centrifuged, 0.5 ml of serum was removed, and radioactivity was measured. In addition to a 0.5-ml aliquot of the serum, 1.5 ml of Con A-Sepharose were added and the mixture was treated in the same fashion as described above. The difference in radioactivity between the pre- and post-Con A-Sepharose samples was also less than 20%.

The serum and Con A-Sepharose-precipitated radioactivity, expressed as percentage of injected dose/ml from each treated blood sample, was plotted on a semilog plot against time. The data were analyzed by a standard curve-peeling technique (20) and the disappearance of radioactivity could be expressed best by a curve that fit two exponentials. For the Con A-Sepharose-precipitated radioactivity, the apparent initial volumes of distribution (vol = $1/A + B$), the half-times ($t_{½}\alpha = ln\ 2/\alpha$, $t_{½}\beta = ln\ 2/\beta$), and the metabolic clearance rates (MCR = $\alpha\beta/(A\beta + B\alpha)$ were calculated (21).

Results

In all experiments, the total radioactivity measured in the serum was slightly greater than the Con A-Sepharose-precipitated radioactivity (Fig. 1), and the latter was used for all calculations.

As noted above, the disappearance of radioactivity, expressed as percentage of dose/ml when plotted against time on a semilog plot, could be expressed best as the sum of two exponentials, as shown in Figure 1. The data for all experiments are shown in Table I and, as noted, were calculated based on a two-exponent model. The mean ± SE vol was 461 ± 78 ml, and the mean $t_{½}\alpha$ and mean $t_{½}\beta$ were 2.5 ± 0.5 hr and 33.1 ± 3.7 hr, respectively. The mean MCR was 559 ± 66 ml/day or 81.4 ± 9.9 ml/day/kg.

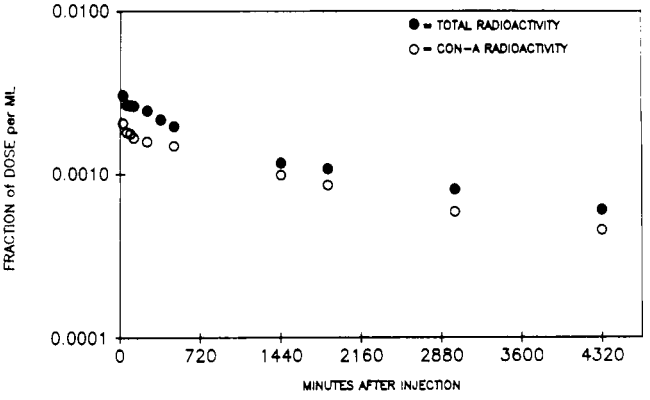

Figure 1. The concentration of radioactivity is expressed as fraction of dose/ml plotted against time in minutes after the injection of [^{125}I]-hSHBG into a rhesus monkey. The concentrations are given as total radioactivity and as concanavalin A-Sepharose-precipitated radioactivity.

Table I. Weights, Volumes of Distribution, and Metabolic Clearance Rates of [^{125}I]-hSHBG in Eight Rhesus Monkeys

Monkey no.	Wt (kg)	Vol (ml)	$t_{½}\alpha$ (hr)	$t_{½}\beta$ (hr)	MCR (ml/day)	MCR (ml/day/kg)
18	6.2	766	1.5	17.7	478	76.5
30	6.0	200	1.2	30.0	700	116.8
31	9.1	728	3.6	29.9	474	52.7
35	6.6	166	2.6	40.0	625	95.2
36	6.6	548	4.6	49.2	349	53.2
40	7.4	183	1.2	20.2	840	113.7
42	9.1	684	1.2	30.0	478	52.7
44	7.2	430	1.7	26.8	811	112.4
Mean	7.0	461	2.5	33.1	559	81.4
±SE	0.3	78	0.5	3.7	66	9.9

Discussion

The metabolism of hSHBG by the rhesus monkey would appear to be slow whether expressed as $t_{½}$ or MCR. This slow metabolism is typical of other glycosylated proteins (9, 10) and appears to be related to the extent of glycosylation (10). Carbohydrate moieties make up 10–20% of hSHBG molecules (1), and this high degree of glycosylation is compatible with the slow metabolism.

The measurements of radioactivity were done in Con A-Sepharose-treated serum, and since the serum is 60% of the blood volume, we calculated that volume would be equivalent to 770 ml of whole blood. The blood volume of the rhesus is in the range of 55–80 ml/kg (22) and the mean weight of our monkeys was 7.0 kg. Thus, the volume was slightly greater than the blood volume, and that is compatible with an initial distribution into the extravascular space. Membrane receptors for hSHBG have been described (23) and early binding to these receptors could account for this initial volume of distribution. It is possible, but unlikely, that the two-compartment model represents different $t_{½}$ as a result of heterogeneity of the administered [^{125}I]-SHBG.

The prolonged $t_{½}$ for the initial, but especially the final, exponentials are reflected in the relatively low MCR expressed as ml/day or ml/day/kg. Assuming that serum was 60% of the blood volume (22, 24), we calculated an MCR of 930 ml of whole blood/day. The cardiac output of rhesus monkeys has been reported to be in the range of 100–300 ml/kg/min or 144 liters/kg/day (24), and that is considerably greater than the MCR of hSHBG that we measured. Since hepatic blood flow is about 20% of cardiac output (25), the MCR of hSHBG would be much less than hepatic blood flow, indicating a low hepatic extraction. This is similar to the results of Suzuki and Sinohara (12), who studied bovine SHBG metabolism in the rat. They reported that 86% of the injected dose was present 10 min after the injection of bovine SHBG into a rat, and our results are in the same range. They also noted that after desialylation of the SHBG, only 1–2% could be found in the plasma. Therefore, the fact that we found so much [^{125}I]-hSHBG in the blood after 20 min would suggest that our iodination procedure did not result in significant desialylation.

Our studies were done in ovariectomized monkeys that had received no recent estrogen replacement. Estrogen administration has been reported to increase the glycosylation of thyroxine-binding globulin (26), but whether the lack of estrogen alters the metabolism of SHBG is not known. It is possible that the MCR of endogenous rSHBG might be different in intact monkeys, and our results using exogenous hSHBG might not reflect such a difference.

Studies in humans have reported fairly rapid, but relatively minor, changes in SHBG concentrations during the menstrual cycle (27) and during the day (28). Also, neither Stanczyk *et al.* (29) nor we (C. Longcope, unpublished data) could detect changes in rSHBG through the rhesus menstrual cycle. Our data indicate that hSHBG has a prolonged $t_{½}$, and that would not be compatible with a rapid change in the mass of hSHBG in the circulation. The changes in the concentration thus may reflect changes in blood volume more than changes in the mass of hSHBG. However, there could be relatively rapid alterations in tissue uptake, or desialylated isoforms of hSHBG could be released episodically that were reactive with the antibody used in the measurements to explain the reported rapid changes. Such release could result in more rapid changes in concentration than would be expected from the form that we used.

Rhesus monkeys have SHBG and their SHBG has a high homology with hSHBG (13). When injected into monkeys, both rSHBG and hSHBG had the same effects on the MCR of testosterone. Thus, our results for the metabolism of hSHBG in monkeys probably are a good reflection of the metabolism of rSHBG in the monkey and hSHBG in the human. However, final confirmation of the latter will require studies done in humans.

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