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Research Article



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# TRANSCORTIN: A CORTICOSTEROID-BINDING PROTEIN OF PLASMA. V. *IN VITRO* INHIBITION OF CORTISOL METABOLISM *

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Elevated concentrations of plasma transcortin have been shown to exist during pregnancy or after the administration of estrogens and to be accompanied by almost concomitant increases in the levels of plasma cortisol (1–4).[1] The latter change has been ascribed to the higher transcortin capacity to bind cortisol. Using these indirect evidences, we have postulated that transcortin-bound cortisol is *1*) biologically inactive and *2*) unavailable for catabolism. The first hypothesis explains the lack of hypercorticism in face of high cortisol levels in pregnancy and in estrogen-treated subjects, and the second hypothesis the slower metabolism of cortisol in the above subjects (4–8). The verification of the first hypothesis has recently been published from our laboratory and is based on the observation that transcortin prevents glycogen deposition in adrenalectomized mice treated with cortisol (9). In this paper we wish to report evidence in support of the second hypothesis, i.e., that transcortin prevents the catabolism of cortisol by human or rat liver homogenates or microsomes.

## METHODS AND MATERIALS

Human liver was obtained at operation and processed immediately at 5° C. Human or rat liver homogenates (10 per cent) were made in 0.05 M phosphate buffer at pH 7.4. For the isolation of microsomes a 10 per cent homogenate in 0.25 M sucrose was prepared by hand using a Potter-Elvehjem homogenizer. After sedimentation of the nuclei and mitochondria, the microsomes were collected as sediment at 104,000 × G. The microsomes were then suspended in phosphate buffer. The homogenates or microsomes were incubated with 1.5 $\mu$g of 4-$C^{14}$-cortisol (13.7 $\mu$c per mg) and one ml of $2 \times 10^{-3}$M TPNH (generated enzymatically immediately before use) (10) in a total volume of 8 ml for 30 minutes at 37.5° C. At the end of the incubation the contents of each flask were extracted twice with 3 volumes of dichloromethane. The extract was filtered, the radioactivity determined (1), and the organic solvent evaporated by a stream of nitrogen. The residue was chromatographed in the Bush B/55 system and scanned in an Actigraph. The radioactivities under the various peaks were determined, and the results are expressed as the following ratio: $F/H_xF$ = cortisol/reduced cortisol. The recoveries of the radioactivity averaged over 80 per cent in the experiments performed.

*Purification of transcortin.* Over 600 ml of plasma from subjects treated with estrogens was dialyzed against 2 volumes of water overnight in the cold room. After adjusting the pH of the dialyzed plasma to 5.0, it was clarified by centrifugation and then placed on a 5 × 35 cm column of diethylaminoethylcellulose (DEAEC), which had been previously conditioned to 0.05 M sodium chloride at pH 5.0. After the plasma had all entered the resin, the column was washed with 0.05 M sodium chloride, pH 5.0, until the optical density of the eluate at 280 m$\mu$ fell to 0.6. A gradient was then started with the following conditions: in the mixing chamber 470 ml of 0.05 M sodium chloride, at pH 5.0; in the reservoir 0.2 M $NaH_2PO_4$-0.075 M sodium chloride, pH 5.0, 1:10; $R_{in} = R_{out} = 6$ to 10 ml per minute; 110 30-ml fractions were collected. The column was run at room temperature (25 to 28° C).

Some protein is removed by lowering the ionic strength and pH, but the bulk of the protein, including the albumin, passes through the column and is discarded before the gradient elution is commenced. When the gradient is started, the blue zone, due presumably to caeruloplasmin, should be at the top of the column; at the completion of the gradient, it should be coming off. The transcortin moves just ahead of the caeruloplasmin.

The eluate was analyzed for protein by absorption at 280 m$\mu$, with ovalbumin as a standard. The transcortin area was localized either by the addition of 4-$C^{14}$-cortisol prior to chromatography and determination of the radioactivity in each tube by gas-flow counting, or by determination of binding by equilibrium dialysis at pH 7.0 (1).

To concentrate the protein, Sephadex G-75 or G-25 was used, 30 g per 600 ml. After standing for 10 min-

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[1] The following abbreviations have been employed—cortisol (F): 11$\beta$,17$\alpha$,21-trihydroxy-4-pregnene-3,20-dione; dihydrocortisol: 11$\beta$,17$\alpha$,21-trihydroxy-pregnane-3,20-dione; tetrahydrocortisol: 3$\alpha$,11$\beta$,17$\alpha$,21-tetrahydroxy-pregnane-20-one; TPNH: reduced triphosphopyridine nucleotide; HSA: human serum albumin.

TABLE I

*The effects of increasing transcortin concentrations on the* $\frac{F}{H_xF}$ *with microsomes from rat liver (1 g)* *

| Transcortin | $\frac{F}{H_xF}$ |
|---|---|
| *mg* | |
| | 0.14 |
| 0.06 | 0.36 |
| 0.12 | 0.35 |
| 0.25 | 0.40 |
| 0.50 | 0.54 |
| 1.00 | 0.78 |
| 2.00 | 1.30 |

* $\frac{F}{H_xF} = \frac{\text{cortisol}}{\text{reduced cortisol}}$.

utes in the cold, the Sephadex was centrifuged down and the supernatant fluid, now one-third of the original volume, was decanted. There was no change in the percentage of binding per mg of protein. This procedure was repeated to reduce the volume again by one-third. Even though the loss of binding ability was small, 70 to 90 per cent of the protein was lost in these two steps. The residues after dialysis against distilled water and after lyophilization were used in both the *in vivo* (9) and the *in vitro* experiments.

## RESULTS

Under the conditions of the experiments outlined above, it was shown that rat liver homogenates or microsomes metabolized $C^{14}$-cortisol primarily to the dihydro and tetrahydro allopregnane derivatives, whereas human liver homogenates or microsomes metabolized $C^{14}$-cortisol primarily to dihydrocortisol and to a smaller extent to tetrahydrocortisol.

Preliminary experiments with homogenates or microsomes indicated that the equivalent of 100 mg to 1,000 mg of liver resulted in substantial reduction of the cortisol molecule. Hence, in most experiments the microsomes from 0.1 to 1.0 g liver were used, since extracts from experiments with microsomes were more easily purified and chromatographed than those obtained from homogenates. Incubation of cortisol without liver resulted in a ratio of infinity, indicating no reduction of the cortisol molecule in the absence of the enzyme source. In Table I the results with rat liver microsomes and increasing amounts of transcortin are shown. It appears that as the concentration of transcortin is increased, less and less of the cortisol is metabolized. In fact, with two mg of transcortin, more $C^{14}$-cortisol was present than the reduced metabolite. In Table II results obtained with different microsomal preparations of rat livers are shown. Even though in each case the ratio differed somewhat, the results demonstrate that substantially less cortisol was metabolized in the presence of transcortin than when equivalent amounts of human serum albumin (HSA) or no added protein are present. The results with human liver microsomes are shown in Table III and are similar to those obtained with rat liver microsomes.

TABLE II

*The effects of transcortin or HSA on the* $\frac{F}{H_xF}$ *with rat liver microsomes**

| Substances added to incubation | Microsomes from rat liver (wt) | | | |
|---|---|---|---|---|
| | 600 *mg* | 1,000 *mg* | 333 *mg* | 839 *mg* |
| Transcortin, 2 *mg* | 0.85 | 0.62 | 1.00 | 1.27 |
| HSA, 2 *mg* | 0.44 | 0.28 | 0.65 | 0.27 |
| | 0.32 | 0.39 | 0.85 | 0.30 |

* HSA = human serum albumin; $\frac{F}{H_xF} = \frac{\text{cortisol}}{\text{reduced cortisol}}$.

In previous studies it was shown that pregnancy plasma or that obtained from patients after estrogen therapy contained more transcortin than normal plasma (1–3). In the next series of experiments plasmas obtained from various subjects were incubated with either human or rat liver microsomes. In each instance less cortisol is metabolized in the presence of plasma from pregnant women or subjects given estrogens than was observed in the presence of normal plasma or no plasma at all (Table IV). Since in the former conditions the plasma cortisol levels are elevated,

TABLE III

*The effects of transcortin or HSA on the* $\frac{F}{H_xF}$ *with human liver microsomes**

| Substances added to incubation | Microsomes from human liver (wt) | | |
|---|---|---|---|
| | 370 *mg* | 100 *mg* | 1,000 *mg* |
| Transcortin, 2 *mg* | >9.0 | 1.0 | 1.0 |
| HSA, 2–25 *mg* | 0.81 | 0.35 | 0.33 |
| | 0.77 | 0.34 | 0.23 |

* HSA = human serum albumin; $\frac{F}{H_xF} = \frac{\text{cortisol}}{\text{reduced cortisol}}$.

TABLE IV

*The effects of plasma (3 ml) from various sources on the* $\frac{F}{H_xF}$

| Source of plasma | Microsomes from liver (wt) | | | | |
|---|---|---|---|---|---|
| | 800 *mg** | 1,625 *mg** | 1,000 *mg** | 600 *mg*† | 415 *mg*‡ |
| Estrogen-treated subjects | 0.90 | 0.68 | 0.99 | 1.10 | 9.8 |
| Pregnancy | 0.62 | 0.93 | 1.20 | 0.90 | 7.2 |
| Normal subjects | 0.46 | 0.45 | 0.33 | 0.42 | 5.5 |
| 25 $\mu$ F | 0.18 | 0.12 | 0.14 | 0.18 | 2.5 |

* Rat liver.
† Normal human liver.
‡ Human cirrhotic liver.

amounts of cortisol ranging from 1 to 25 $\mu$g were incubated with liver and resulted (except for the cirrhotic liver) in almost complete reduction of the steroid. This would indicate that under the conditions of the experiments the endogenous cortisol would not be sufficient to interfere with the reduction of the $C^{14}$-cortisol by the microsomes. Incubation with 25 to 100 mg of human serum albumin gave results which, in essence, did not differ from those seen when protein was absent from the incubation medium.

## DISCUSSION

The prevention of cortisol-induced glycogen deposition in the livers of adrenalectomized mice by the administration of transcortin supported our hypothesis that transcortin-bound cortisol appears to be biologically inactive (9). Implicit in this hypothesis are two postulates: *1*) that the biologically effective levels of cortisol in the body are not related to the total plasma concentration of the steroid, but to that which is not bound to transcortin, and *2*) that the transcortin-bound cortisol should not be available for catabolism.

The latter biologic potential of transcortin has been demonstrated in the experiments presented. In previously published investigations it was shown that cortisol is metabolized at a greatly reduced rate in subjects with high concentrations of plasma transcortin (4, 6–8). These results were interpreted as indicating that the much slower metabolism of cortisol was due to its being bound to transcortin and thus rendered unavailable for catabolism by the liver. The *in vitro* experiments presented in the present paper lend further support to the hypothesis. The inability of rat or liver homogenates or microsomes to reduce the cortisol molecule efficiently in the presence of transcortin (either in concentrated form or in transcortin-rich plasma) points to the ability of transcortin to bind cortisol much more strongly than the enzyme systems in the liver. On the other hand, human serum albumin was not capable of competing with the liver enzyme systems for cortisol, indicating a low affinity of the albumin for the steroid, as has been previously reported (1).

Even though the *in vitro* experiments presented indicate successful competition by transcortin with the liver enzyme systems for cortisol, conditions prevailing *in vivo* may possibly accentuate the effects of the higher affinity of transcortin for cortisol. At present little is known about the pathways or means by which cortisol enters the hepatic cells and the events leading to the catabolism of the steroid. It is possible that only the unbound cortisol is available for catabolism, and hence, in the presence of increased transcortin concentrations in the plasma relatively less of the steroid may be available for metabolism. In addition, a certain amount of energy of transfer may be involved in the removal of cortisol from transcortin and the transport of the steroid across the hepatic cellular membrane to the reductive intracellular enzyme system. If in the presence of increased transcortin concentration, the amount of energy of transfer remained constant, it is possible to visualize another factor responsible for the slower metabolism of plasma cortisol. With a constant amount of energy of transfer available, relatively less of cortisol would be removed from transcortin, and hence, less cortisol would be metabolized.

The successful competition by transcortin for

cortisol with the reductive enzymes of the liver *in vitro* would seem to indicate that transcortin probably has a higher affinity for the steroid than that shown by any other human protein tested to date. This differential in affinity between transcortin and the reductive enzymes is probably accentuated *in vivo* by the intracellular locations of the latter and magnified by the presence of increased transcortin concentrations.

## SUMMARY

In an *in vitro* system, employing either human or rat liver microsomes, transcortin competes efficiently for cortisol with the enzyme systems responsible for the reduction of the steroid, resulting in lesser metabolism of cortisol in the presence of transcortin. The addition of transcortin-rich plasma (pregnancy, estrogen-treated subjects) to the incubation gave results similar to those obtained with purified transcortin. The partial purification of the latter is described. The results obtained bear on the interpretation of the effects of transcortin on cortisol metabolism *in vivo*.

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