^{3}H-CORTISOL RADIOACTIVITY IN HEPATIC SMOOTH ENDOPLASMIC RETICULUM[1]

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SUMMARY

After a lag of 15 minutes, the microsomal fraction of liver rapidly accumulates injected cortisol radioactivity at a rate parallel to the liver homogenate. The hormone binds mainly to the smooth membrane fraction, poorly to the rough membrane fraction and virtually not at all to the ribosomes. The bound radioactivity is not released by extensive water washing of either membrane fraction but can be extracted with n-butanol. Anionic metabolites, electrophoretically similar to those found in the cytosol, are formed with time on the smooth membrane. The data suggest hormone binding to metabolizing enzymes of the smooth membrane and possibly to other structural sites which may be obscured by ribosomes in the rough membrane.

The subcellular distribution of cortisol radioactivity in rat liver, in the order of concentrating ability, is the cytosol > microsomal fraction > nuclear fraction (1). The fate of radioactive cortisol in the cytosol fraction has been investigated in detail (2-5) but the nature of the accumulation in the microsomal compartment has received little attention. Recently, evidence from electron microscopy has shown that as early as

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15 min. after cortisol injection, pronounced changes occur in the hepatocyte endoplasmic reticulum, specifically, in the conversion of parallel stacks of rough endoplasmic reticulum to vesiculated smooth membranes (6). Changes in other subcellular structures in response to glucocorticoids are not observed by this technique until several hours or days later (7-10). These results prompted experiments on the localization and nature of the cortisol radioactivity in the microsomal compartment.

MATERIALS AND METHODS

Male, adrenalectomized Wistar strain rats weighing 80 to 120 g were obtained from the Charles River Breeding Laboratories. The animals were fed Purina rat chow, a 1% NaCl drinking solution and were used for experiment within 10 days of adrenalectomy. Cortisol-1,2-^{3}H (New England Nuclear; specific radioactivity, 125 mC/mg) was dried in a stream of filtered nitrogen and resuspended in sterile saline for intraperitoneal injection of from 2.1 to 2.8 x 10^{8} dpm (5). Animals were decapitated at various times following injection. After perfusion *in situ* with chilled sterile saline by way of the portal vein, livers were excised, chilled in ice, washed with 10 to 20 ml sterile saline, blotted, weighed and homogenized in 3 volumes 0.88 M sucrose. Rough and smooth endoplasmic reticulum were isolated from the homogenate by the method of Fouts (11). The isolated membranes were resuspended in 10 ml water and centrifuged in a Beckman L-2 preparative ultracentrifuge for 90 min. at 100,000 x g. This washing was repeated twice and the rough and smooth membranes were finally suspended in 5.0 ml water. Rough and smooth membrane fractions were extracted three times with two volumes of n-butanol or ethyl acetate. Each solvent extraction was for 5 minutes followed by centrifugation for 5 min. at 0 to 1°C. The extracts were pooled, dried by flash evaporation and dissolved in 0.3 ml of n-butanol. The concentrates were spotted on Whatman 3 MM paper (60 x 2 cm) and dried in a stream of cool air. A

saturated solution of picric acid and caffeine was used to define extent of the run and electroosmosis. R_m values are reported relative to the migration of picric acid towards the anode. Strips were subjected to electrophoresis at 1700 volts in 0.05 M sodium tetraborate buffer, pH 9.2, as described previously (3). Total ribosomes or free ribosomes were prepared from a post-mitochondrial supernatant by the method of Blobel and Potter (12). Radioactivity of aqueous samples was determined in a dioxane based fluor-Cab-o-sil system (13). Electrophoretic strips were segmented into 1.5 x 2 cm and the radioactivity of each segment was measured in 5 ml of a toluene-fluor (Spectrofluor, Amersham-Searle) solution using a Nuclear-Chicago Model 723 scintillation spectrometer. Efficiency was determined by the channels ratio technique.

RESULTS AND DISCUSSION

Cortisol has been shown to be accumulated rapidly in the microsomal fraction (1) and to cause aggregation of polysomes within 4 hours after injection (14,15). We tested the hypothesis that the hormone was bound by the ribosomes themselves. Total ribosomes or free ribosomes failed to bind the hormone significantly in in vitro or in vivo experiments where binding was measured by ultrafiltration. This negative result turned our attention to the membranes themselves. The total membrane fraction concentrates the injected cortisol very rapidly following a lag between 5 and 15 min. and a steady state is reached between 30 and 45 min. after injection. After 15 min. the rate of accumulation in the total membrane fraction parallels the rate in the whole liver. Separation of the microsomal fraction into smooth and rough fractions shows a significant difference in uptake (Fig. 1). The smooth fraction concentrates radioactivity very rapidly from 15 to 45 min. postinjection. The rough endoplasmic reticulum, on the other hand, remains essentially unchanged in its uptake throughout the experimental period. Although both fractions

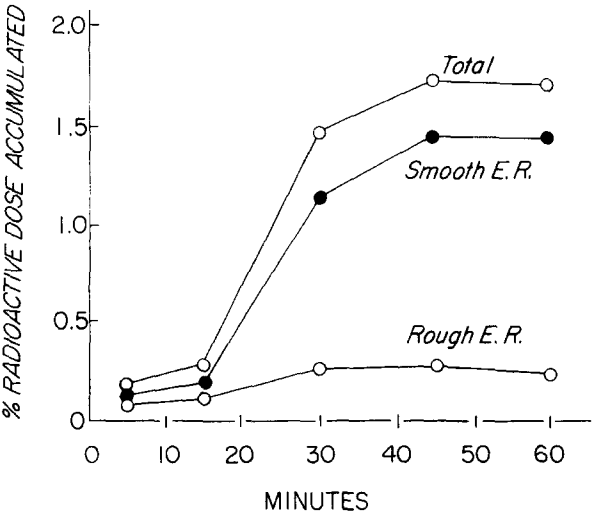

Fig. 1 Time-course of accumulation in vivo of cortisol radioactivity in isolated smooth and rough endoplasmic reticulum membranes.

function similarly in the first 15 min. postinjection, the smooth fraction becomes much more active in the ensuing period. Extraction of membrane fractions with n-butanol (Fig. 2) or with ethyl acetate showed that both cortisol and anionic metabolites are bound to the smooth membranes, whereas throughout the time period (5-60 min.) the rough reticulum contained virtually no metabolites. At 15 min. the washed smooth reticulum contained nearly 0.2% of the dose and the washed rough reticulum contained slightly more than 0.1% of the dose. At 45 min. the washed smooth fraction contained 1.5% of the dose whereas the washed rough fraction had only about 0.3% of the dose. The radioactivity associated with the rough membrane maintained the same position as the cortisol marker during high voltage electrophoresis. Ultimately four anionic metabolite peaks were observed in the smooth fraction. These had $R_{m\ picrate}$ values of 0.26, 0.60, 0.80 and 0.92 (Fig. 2). These values agree very closely with those of cortisol metabolites occurring in the cytosol fraction (5) the two most anionic of which have been designated in preliminary experiments as sulfate derivatives.[1]

[1] S. Singer and G. Litwack, unpublished experiments

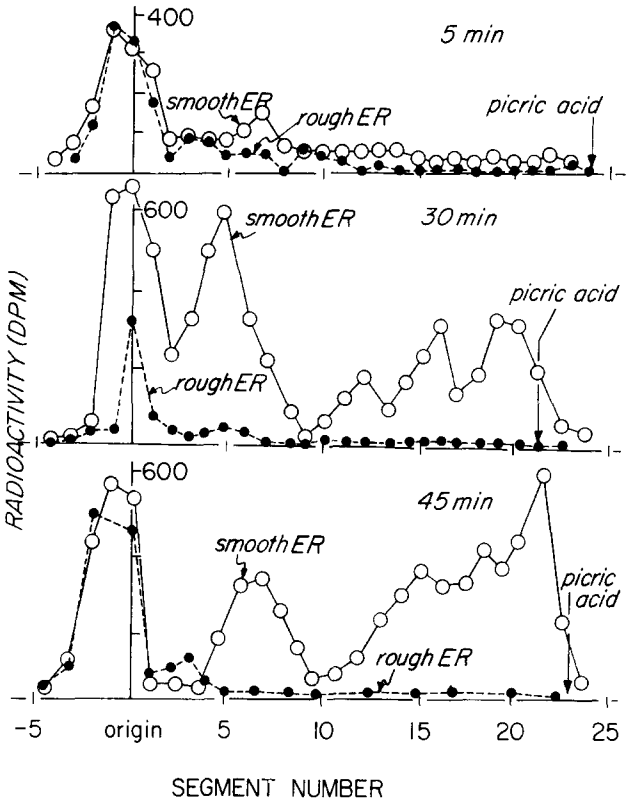

Fig. 2 High voltage electrophoretograms of n-butanol extractable radioactivity from smooth and rough endoplasmic reticulum showing qualitatively the distribution of hormone and metabolites.

Five min. after injection, 5.7% of smooth membrane radioactivity was in the form of slowly migrating metabolite ($R_{m\ picrate}$, 0.26). After 15 min. (data not shown) this metabolite represented 36% and 6.5% was in the form of more anionic metabolites ($R_{m\ picrate}$ 0.88). At 30 min., the slow metabolite was 46% of the radioactivity of the ionophoretogram with the more anionic metabolites increasing to 20%. The more highly charged metabolites resolved into 3 peaks with $R_{m\ picrate}$ values of 0.60, 0.80 and 0.92. By 45 min. postinjection the slow peak had decreased to 17% while the three more negatively charged compounds totaled 60% of the radioactivity. One hour after injection (data not shown) the distribution of radioactivity was similar to that of the 45 min. period. Kinetics of the disappearance of

the slow fraction as the more anionic fractions accumulate suggest a precursor-product relationship.

The smooth and rough membranes have about the same protein concentration (16). Although the protein composition is qualitatively similar in the two microsomal membrane fractions, some proteins appear to be present in different amounts and specific proteins may be unique to each fraction (17). Both membrane fractions have a similar phospholipid-protein construction (18). The affinity of the hormone for the smooth membranes probably reflects the content of metabolizing enzymes as well as structural binding sites. Although ribosomes themselves do not bind the hormone, their presence on the membrane may block the site of attachment of the hormone. If this is the case, an extrapolated view might be one in which the steroid binds (functionally?) to or near the ribosome binding site on the membrane.

REFERENCES

1. Litwack, G., Sears, M.L. and Diamondstone, T.I. J. Biol. Chem. 238, 302 (1963).
2. Litwack, G., Fiala, E.S. and Filosa, R.J. Biochim. Biophys. Acta 111, 569 (1965).
3. Fiala, E.S. and Litwack, G. Biochim. Biophys. Acta 124, 260 (1966).
4. Litwack, G. and Baserga, R. Endocrinology 80, 774 (1967).
5. Morey, K.S. and Litwack, G. Biochemistry, in press.
6. Rancourt, M.W. and Litwack, G. Expt. Cell Res. 51, 413 (1968).
7. Gustafsson, R.G. and Afzelius, B.A. J. Nat'l Cancer Inst. 30, 1045 (1963)
8. Orrenius, S. and Ericsson, J.L.E. J. Cell Biol. 28, 181 (1966).
9. Weiner, J., Loud, A.V., Kimberg, D.V. and Spiro, D. J. Cell Biol. 37, 47 (1968).
10. Kimberg, D.V., Loud, A.V. and Weiner, J. J. Cell Biol. 37, 63 (1968).
11. Fouts, J.R. Biochem. Biophys. Res. Commun. 6, 373 (1961).
12. Blobel, G., and Potter, V.R. J. Mol. Biol. 26, 279 (1967).
13. Weinstein, A., Medes, G. and Litwack, G. Anal. Biochem. 21, 86 (1967).

14. Cammarano, P., Chinali, G., Gaetani, S. and Spadoni, M.A. Biochim. Biophys. Acta 155, 302 (1968).

15. Sidransky, H., Wagle, D.S. and Verney. E. Lab. Investigation 20, 364 (1969).

16. Bergstrand, A. and Dallner, G. Anal. Biochem. 29 351 (1969).

17. Schnaitman, C.A. Proc. Nat'l Acad. Sci. 63, 412 (1969).

18. Arias, I.M., Barrell, D. and Schimke, R.T. J. Biol. Chem. 244, 3303 (1969).