Ligandin Content of Normal and Carcinogen-Treated Rat Tissues

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Key Words

Ligandin
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Abstract

Ligandin was detected by immunofluorescence in tissue sections and determined by immunoquantitation in the cytosols of the liver, kidney and testes of normal and carcinogen-treated rats. Ligandin was not detected by either of these procedures in normal or carcinogen-treated rat lung, spleen, brain, and skeletal or cardiac muscle.

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Introduction

Ligandin has been purified from rat and human liver and rat kidney and is a basic protein with 2 apparently identical monomeric units [1–3]. In the rat, ligandin is identical with hepatic azocarcinogen binding protein [2, 4], estrogen binder [2, 4] and GSH transferase B [5]. This protein covalently binds metabolites of the carcinogens N, N-dimethylaminoazobenzene and 3-methylcholanthrene [6]. Therefore, monospecific precipitating antibodies were prepared against ligandin (1) to determine in the rat the cellular localization by immunofluorescence of ligandin in normal and 3'-methyl-p-di-methylaminoazobenzene and methylcholanthrene-treated tissues (liver, kidney, testes, lung, brain, spleen, cardiac and skeletal muscle), and (2) to quantitate by radial immunodiffusion the ligandin content of the cytosols of these same normal and carcinogen-treated tissues. This study describes the results of these experiments.

Materials and Methods

Inbred male CDF Fischer rats (Charles River Breeding Laboratories, Wilmington, Mass.) 6–8 months old and weighing about 300 g were used. The carcinogens, 3'-methyl-/?-dimethylaminoazobenzene

(3'-Me-DAB) and 3-methylcholanthrene (MC) were purchased from Eastman Organic Chemicals, Rochester, N.Y. Pure ligandin was obtained through the courtesy of Dr. N. M. Bass, NCT-MRC Liver Research Group, Department of Medicine, University of Cape Town, Republic of South Africa; Dr. B. Ketterer, Middlesex Hospital Medical School, London, England, and Dr. G. Litwack, Temple University School of Medicine, Philadelphia, Pa.

Antisera were raised in rabbits against pure ligandin as previously described [7]. The carcinogens were dissolved in cotton seed oil (20 mg/ml) and administered by stomach tube to rats (20 mg/100 g) as was done previously [8]. The 3'-Me-DAB-treated rats were sacrificed after an overnight fast at 41 h after treatment when there is maximum incorporation of this carcinogen

into liver proteins [8]. The MC-treated rats were sacrificed after an overnight fast 17 h after treatment the time at which MC has near optimal inducing effect on drug metabolizing enzymes [9]. Sodium barbital was dissolved (10 mg/ml) in 0.15 M NaCl and the rats were injected intraperitoneally with a dose of 10 mg/100 g of rat once a day for 3 days and were killed 24 h after the last injection. Also these same barbital-treated rats were given 3'-Me-DAB (20 mg/100 g) by stomach tube 42 h before sacrificing. The rats were fasted overnight before killing under ether anesthesia. Tissue cytosols of the rat liver, brain, lung, spleen, testes, kidney, heart and skeletal muscle were prepared by differential cen-trifugation of 0.25 M sucrose homogenates [10]. Due to the small size of some of the rat organs, similar organs from each of 2 rats were pooled for each experiment.

The immunoquantitation of ligandin in the various tissue cytosols and the localization of this cytosol protein by fluorescence microscopy was carried out as previously detailed [7].

Specificity of the immuno-fluorescent conjugate was verified by using the following control sections of liver and other tissues: (1) untreated, (2) incubated with sera of normal rabbits, (3) incubated with antisera from which anti-

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bodies against ligandin were removed with pure ligandin or cytosols of liver obtained by centrifugation, and (4) lack of staining in tissues (muscle, spleen, lung) which do not contain ligandin. Also, blocking of fluorescence was accomplished by prior incubation of liver with 1 % goat antirabbit IgG globulins not conjugated with fluorescein.

Results and Discussion

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Ligandin was shown to be present by the fluorescence antibody procedure in the cytoplasm of rat liver, kidney and testes, but this protein could not be detected in the cells of brain, spleen, lung and cardiac or skeletal muscle. Using the same procedure, Fleischner et al. [11] found that ligandin was present in the liver, kidney and small intestine of rats, hamster and man, but this protein was not detected in any other organ of these species. On the other hand, immunofluorescent activity of ligandin was observed in the periportal zones of rat liver, kidney, small intestine, ovary and testes by Bannikov et al. [12] and as indicated above, in the testes in the studies reported here. While the differences between the studies reported by Bannikov et al. [12], together with those reported in this paper and those of Fleischner et al. [11] cannot be resolved at this time, such differences cannot be explained by a lack of specificity of immunofluorescent conjugates used in this study.

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 Testes
 Kidney

Fig. 1. Effect of carcinogens on the ligandin content (mg/100 mg cytosol) of normal and carcinogen-treated rat liver, kidney and testes. The letters a, b, c and d represent respectively organ ligandin levels of normal, 3'-Me-DAB, MC and 3'-Me-DAB-barbital-treated rats. Columns, means of 4 rats/group except control testes which had 3 rats; bars, SD. * Significant difference from control group of the liver ($p > 0.05$) and kidney ($p > 0.01$) of the carcinogen-treated rats. ** Significant difference ($p < 0.001$) from the barbital-3'-Me-DAB-treated testes of the control, 3'-Me-DAB- and MC-treated testes.

Ligandin was determined in some normal and carcinogen-treated tissues by immunoquantitation [7]. Liver cytosol contained the largest amount of ligandin (fig. 1) and the content of this protein was decreased significantly in the liver by the administration of 3'-Me-DAB (b), MC (c) or barbital and 3'-Me-DAB (d). In experiments comparable to those as shown in figure 1, barbital increased significantly the binding of DAB to DNA and microsomal protein of rat liver [9]. Kidney cytosol of normal rats contained 20% less ligandin than did liver, and the carcinogens decreased significantly the kidney cytosol level (fig. 1: kidney – b-d). Testes had the lowest level of ligandin, and the amount of this protein was increased significantly in the barbital-3'-Me-DAB-treated rats (fig. 1: testes – d). Ligandin was not detected in the cytosol of rat brain, spleen, lung and cardiac or skeletal muscle. The average ligandin values (mg/100 mg cytosol) of 4.1 for liver and 2.8 for kidney are quite comparable to those reported by Fleischner et al. [11] of 4.4 for liver and 2.2 for kidney. Bannikov et al. [12], employing an immunochemical quantitative procedure, showed that water extract of rat liver, small intestine and kidney had comparable amounts of ligandin whereas low concentrations of this protein were found in water extracts of ovary and testes. Bannikov et al. [12] did not detect ligandin in water extracts of many rat tissues including spleen, lung, pancreas, myocardium, skeletal muscle and brain. The amount of ligandin determined semiquantitatively in the cell sap of rat liver was found to be significantly reduced in primary hepatomas induced by several different carcinogens [13].

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