RELEASE OF DNA FROM RAT HEPATOCYTE NUCLEI AFTER TREATMENT WITH HEPARIN

O. E. Demidenko and S. E. Tsvetkova

UDC 612.351.1:612.398.145.1-06:615.273.55

The effect of heparin on protein fractions and DNA of isolated hepatocyte nuclei from Wistar rats was studied. Biochemical and cytospectrophotometric tests showed that after exposure to 0.05% sodium heparinate solution 95% of histones were blocked; heparin had no action on the nonhistone proteins of the cell nucleus. Histone blocking was connected with release of DNA from the nuclei into the incubation medium; the quantity of DNA liberated was a linear function of the incubation time.

KEY WORDS: heparin; isolated nuclei; protein fractions; DNA.

In the cytoplasm of functionally active cells DNA of nonmitochondrial nature is found [3, 7]. Little is known of the mechanism of appearance of this DNA. A marked increase in the concentration of microsomal DNA in the cytoplasm of mouse liver cells has been shown [1] under the influence of so-called low-molecular-weight liver compounds. At the same time, there is evidence that cytoplasmic DNA is nuclear in origin and that its appearance is promoted by separation of histones from the DNA [3].

In this investigation the effect of heparin on protein fractions and DNA of isolated rat hepatocyte nuclei was studied.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar albino rats weighing 180-200 g. Liver tissue of three or more animals was pooled for the investigation. The isolated nuclei [5] were suspended in 0.14 M NaCl (pH 7.2) to which a 0.1% solution of sodium heparinate had been added to produce a 0.05% solution. The suspension was incubated at 20°C for 2 to 10 min. Control nuclei were kept in 0.14 M NaCl without heparin. Histones were extracted from the control nuclei and the heparin-treated nuclei with 0.25 N HCl, and protein was determined by Lowry's method [8]. The DNA content in the nuclei and supernatant fluid was determined by Burton's method [4].

For the cytophotometric measurements films of control and heparin-treated nuclei were prepared, fixed with 10% formalin, and stained for DNA and for total and nonhistone protein [2]. Altogether 50 nuclei from each preparation were measured photometrically with the CITO-2 scanning and integrating cytospectrophotometer.

EXPERIMENTAL RESULTS

A small quantity of DNA (1%), which did not change in the course of incubation, was found in the supernatant of the control nuclei. After treatment with heparin, DNA appeared in the supernatant and its concentration (relative to the nuclear residue) increased in the course of incubation with heparin (Fig. 1).

The control nuclei contained histones in the proportion of 15 pg per nucleus. On treatment of the nuclei with heparin for 2, 5, and 10 min the histones bound with the heparin and the hydrochloric acid extract contained 8.17, 4.75, and 5.50%, respectively, of their control amounts. The difference between the amounts of

Laboratory of Chemistry of the Cancer Cell, Latvian Scientific-Research Institute of Experimental and Clinical Medicine, Ministry of Health of the Latvian SSR, Riga. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 5, pp. 551-553, May, 1977. Original article submitted August 27, 1976.

This material is protected by copyright registered in the name of Plenum Publishing Corporation, 227 West 17th Street, New York, N.Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for $7.50.

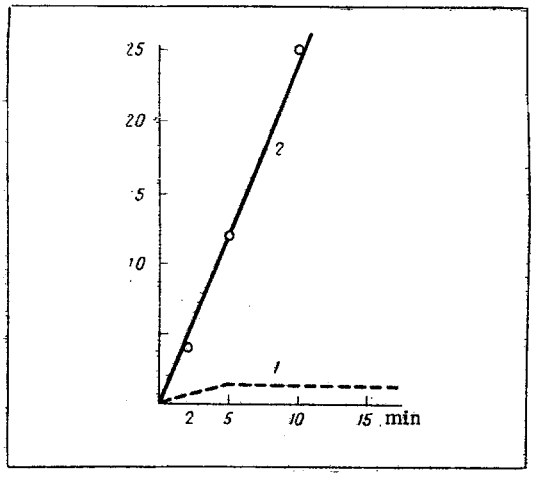

Fig. 1. Dynamics of release of DNA into supernatant during treatment of rat hepatocyte nuclei with 0.05% solution of sodium heparinate: 1) control nuclei; 2) nuclei treated with heparin. Abscissa, incubation time (in min); ordinate, percentage release of DNA into incubation medium.

TABLE 1. Effect of Heparin on Content of DNA and Total and Nonhistone Proteins in Isolated Nuclei of Rat Liver Based on Results of Cytospectrophotometry (mean measurements of optical density for 50 nuclei given in conventional units)

Experiment No.	Total proteins			Nonhistone proteins			DNA		
	control	heparin	% decrease in optical density	control	heparin	% decrease in optical density	control	heparin	% decrease in optical density
1	2028	1070	47,24	1243†	1395†	—	1043	849	18,6
2	2039	1122	44,98	1669†	1579†	—	1085	939	13,5
3	2309	1234*	46,56	1127†	1278†	—	1098	966	12,0
4	2311	1108*	52,06	1199	1372	—	1123	953	15,1
5	1834	1194	34,9	1553†	1595†	—	1129	888	21,4
6	2403	1152*	52,06	1092	1113†	—	1385	1111	19,8
7	2131	1184*	44,44	1106†	1217†	—	1333	1040	22,0
Mean	2151±84,8	1152±43,0	46,03	1284±60,5	1364±61,8	—	1171±38,8	965±32,7	17,5

*Difference between "total proteins–heparin" and "nonhistone proteins–control" not significant.

†Differences between "nonhistone proteins–control" and "nonhistone proteins–heparin" not significant.

bound histones after treatment of the nuclei for 5 and 10 min was not statistically significant; it thus appears that blocking of histones reached a maximum after incubation with heparin for 5 min.

On cytophotometry the optical density of the nuclei stained for DNA fell by 17.5% during the 5 min after heparin treatment (Table 1). These findings are close to the decrease in DNA in the nuclear residue observed in biochemical determinations (12%). The optical density of the nuclei stained for total proteins fell by 46% under the same conditions; the differences in the optical density of the nonhistone proteins in the control and of the total proteins after heparin treatment and also of the nonhistone proteins in the control and nonhistone proteins after heparin treatment were not significant in most experiments (Table 1).

The results of this investigation indicate specificity of the action of heparin relative to histones. Under the experimental conditions chosen an almost total histone block quickly developed; nonhistone proteins of the cell nuclei were not affected under these circumstances. After the blockade of the histones, diffusion of DNA into the incubation medium was observed; the release of DNA from the nuclei was a linear function of the incubation time and was confirmed by biochemical and cytophotometric determinations of DNA in the nuclei

and in the supernatant. A similar pattern also was observed by Ide [6] after dissociation of deoxyribonucleoproteins by x rays in experiments in vivo. The results now obtained are in agreement with the view that one of the functions of histones is to determine the nuclear localization of DNA [3].

LITERATURE CITED

1. S. Yu. Abakumova, N. G. Kutsenko, and M. I. Lerman, Dokl. Akad. Nauk SSSR, 227, 742 (1976).
2. O. E. Demidenko, Izv. Akad. Nauk Latv. SSR, No. 11, 69 (1975).
3. Ya. G. Érenpreis, Usp. Sovrem. Biol, 81, 37 (1975).
4. K. Burton, Biochem. J., 62, 315 (1956).
5. A. Girolamo, E. Henshaw, and H. H. Hiatt, J. Mol. Biol., 8, 479 (1964).
6. H. Ide, Annot. Zool. Jpn., 42, 142 (1969).
7. M. T. Kuo, W. Meinke, and G. F. Saunders, Proc. Nat. Acad. Sci. USA, 72, 5004 (1975).
8. O. H. Lowry et al., J. Biol. Chem., 193, 265 (1951).

RESISTANCE OF DIFFERENT FORMS OF CYTOCHROME P-450 OF RAT LIVER TO FACTORS DESTABILIZING THE MICROSOMAL MEMBRANE

O. A. Gromova, N. E. Zakharova, V. V. Lyakhovich, and I. B. Tsyrlov

UDC 612.351.11.014.46

The effect of factors destabilizing the membrane of the liver microsomes on the spectral properties of cytochrome P-450 (P-448) was investigated in intact rats and rats receiving phenobarbital (PB) or 3-methylcholanthrene (MC). Considerable resistance of microsomes induced by PB and MC to enzymic and nonenzymic peroxidation of polyunsaturated fatty acids of membrane phospholipids was discovered. A clear difference was shown in the sensitivity of cytochrome P-448 and cytochrome P-450 of intact rats and rats receiving PB to in vitro treatment with sodium deoxycholate. The results indicate structural changes in the microsomal membrane during induction by PB and MC, which are two different types of inducers of the monooxygenases of the liver.

KEY WORDS: cytochrome P-450; induction; peroxidation of lipids; deoxycholate.

The system of monooxygenases, responsible for the metabolism of drugs, toxins, and hormones in the liver, is a membrane-bound polyenzymic complex with the property of reversibly increasing its activity in the response to administration of various xenobiotics to animals [4, 11]. Phenobarbital (PB) and the polycyclic hydrocarbon 3-methylcholanthrene (MC) are representatives of two large chemically heterogeneous groups of inducers. The basic differences between the properties of the components of the above-mentioned enzyme system, when induced by PB and MC, have now been sufficiently well described [2, 4, 11], but little information is yet available on the organization of the membrane structures of the microsomes and their mutual relations with the enzymic complexes.

Cytochrome P-450 is the terminal region of the electron transport chain in the microsomes and lies in the hydrophobic zone of the membrane [1, 12]; its action depends on the character of the hydrophobic environment, in the creation of which a leading role is played by phospholipids.

Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk.

(Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 5, pp. 553-555, May, 1977. Original article submitted August 13, 1976.

This material is protected by copyright registered in the name of Plenum Publishing Corporation, 227 West 17th Street, New York, N.Y. 10011. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording or otherwise, without written permission of the publisher. A copy of this article is available from the publisher for $7.50.