BIOPHYSICS AND BIOCHEMISTRY

Effect of Benz(a)pyrene and Constant Light Exposure on Rat Liver Lysosomes and Biliary Excretion of Lysosomal Enzymes

A. B. Pupyshev, E. M. Gutina, R. G. Fedina, S. V. Michurina, A. V. Shurlygina, and L. V. Verbitskaya

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 139, No. 1, pp. 40-43, January, 2005
Original article submitted January 19, 2004

Threefold administration of benz(a)pyrene in a dose of 20 mg/kg markedly stimulated biliary excretion of lysosomal enzymes from rat liver without signs of lysosomal damage. Constant light exposure induced changes attesting to functional activation of the lysosomal apparatus in liver cells and inhibited constitutive biliary excretion of lysosomal enzymes. Combined treatment decreased, but not abolished the stimulatory effect of benz(a)pyrene on vesicular transport of lysosomal enzymes to the bile.

Key Words: *benz(a)pyrene; light-induced desynchronosis; liver; bile; lysosomes*

Polycyclic aromatic carbohydrates, including benz(a)pyrene (BP), possess carcinogenic activity. In liver lysosomes BP is converted into reactive metabolites capable of inducing oncogenic transformation [14]. However, the most part of this preparation is conjugated with glucuronate and released into the bile [9]. BP is partially accumulated in lysosomes, incorporates into the lysosomal membranes, and cause their labialization [3,5]. Little is known about the influence of BP on functional activity of lysosomes, in particular, on excretion of the lysosomal content into bile, although this mechanism underlies removal of intracellular carcinogens. Much attention was paid to the ability of BP to induce carrier proteins determining multidrug resistance [13]. The release of lysosomes into bile and transfer of carrier proteins to canalicular membranes are realized via vesicular transport from the Golgi apparatus to the apical region of hepatocytes [10]. These vesicular flows have the same directionality. Therefore, it was interesting to study the effect of BP on biliary excretion of lysosomal enzymes (LE). Activity of lysosomes and vesicular transport in bile are characterized by diurnal biological variations [8,12] and depend on changes in light/dark regimen (*e.g.*, constant illumination, CI). This exposure can be considered as a stress factor modulating the reaction of liver cell vesicles to BP [7].

Here we studied the effect of BP on the state of liver lysosomes and biliary excretion of the lysosomal content. We also determined the sensitivity of this process to changes in the light/dark cycle (24-h light exposure).

MATERIALS AND METHODS

Male Wistar rats (n=52) weighing 180-220 g were divided into 2 groups. Group 1 rats were exposed to constant 24-h illumination for 2 weeks (light-induced phase shift). Group 2 rats were kept under the natural light/dark conditions. Each group was divided into 3 subgroups. Subgroup 1 rats served as the control. Sub-

Central Research Laboratory, Novosibirsk State Medical Academy. ***Address for correspondence:*** apupyshev@mail.ru. A. B. Pupyshev

0007-4888/05/1391-0034

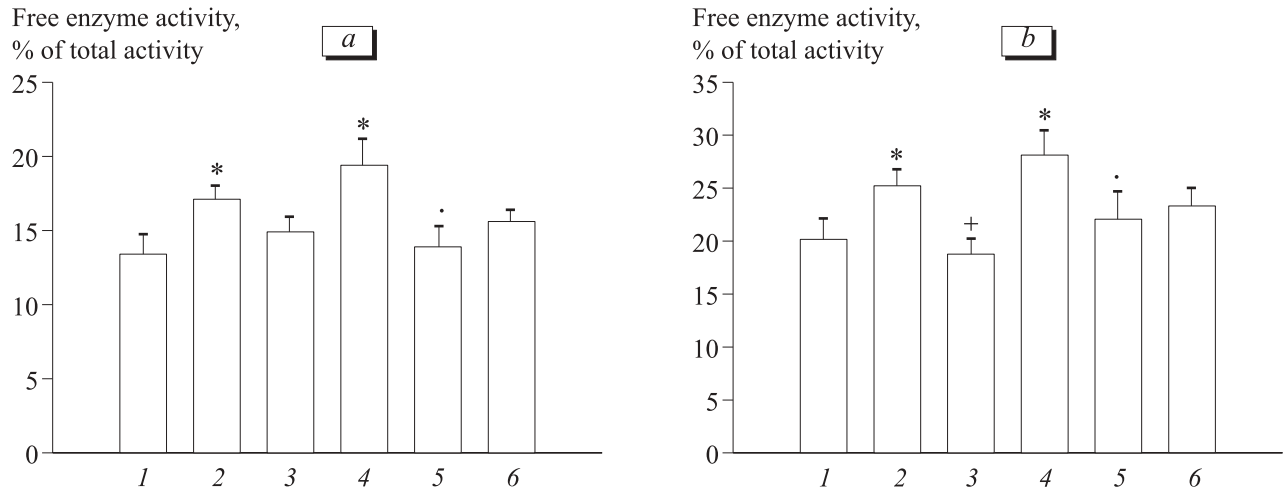

Fig. 1. Activity of free β-galactosidase (*a*) and acid phosphatase (*b*) in rat liver homogenate after administration of benz(a)pyrene and exposure to constant light (CI). Here and in Figs. 2 and 3: control (*1*), olive oil (*2*), benz(a)pyrene (*3*), CI (*4*), CI+olive oil (*5*), and CI+benz(a)pyrene (*6*). $p<0.05$: *compared to the control; +compared to olive oil; °compared to CI.

group 2 rats received intraperitoneal injections of olive oil (1 ml/kg) on days 14, 15, and 16. Subgroup 3 rats were intraperitoneally injected with BP in olive oil on days 14, 15, and 16 (single dose 20 mg/kg, total dose 60 mg/kg). The bile duct was cannulated on the next day under ether anesthesia. Two samples of bile were collected for 5 min. The rats were decapitated, and the blood was taken to obtain the plasma.

The liver was perfused with cold isotonic sucrose. The liver homogenate (20%) was prepared in 0.25 M sucrose and 1 mM ethylenediaminetetraacetic acid (pH 7.4). Activities of acid phosphatase and β-galactosidase in this homogenate were measured using 2-glycerophosphate and 4-nitrophenyl-β-D-galactopyranoside as the substrates, respectively. Activities of β-galactosidase and N-acetyl-β,D-glucosaminidase in the bile and plasma were measured using 4-methylumbellipheryl-β,D-galactopyranoside and 4-methylumbellipheryl-N-acetyl-β,D-glucosaminide as the substrates, respectively [2].

The results were analyzed by Student's *t* test.

RESULTS

Published data show that free LE activity is sensitive to various physiological factors [1] and membrane damage. However, our study revealed only little changes in free LE activity (Fig. 1). BP did not seriously damaged lysosomes in rat liver homogenate. It should be emphasized that free enzyme activity significantly increased under conditions of CI (by 6-8% of total enzyme activity). CI in combination with administration of BP had no effect on enzyme activity. These data show that the reaction of lysosomes to CI is typical of lysosomal activation [1] and manifested in stimulation of heterolysosome formation (secondary lysosomes), which limits the increase in free enzyme activity. BP had no additional destabilizing effects.

No significant changes in LE activity were observed in the plasma (Fig. 2). We revealed only an increase in β-galactosidase activity in rats receiving olive oil and exposed to CI. The increase in plasma LE concentration is accompanied by a significant increase in free enzyme activity in the liver and usually produces vital damage to liver cell lysosomes. Our study did not reveal parallel increase in these indexes. Therefore, signs of labilization of the lysosomal membrane during CI refer only to changes in functional activity of lysosomes. This is manifested in variations in the populational composition of lysosomes (increased formation of secondary lysosomes, activation of autophagocytosis and heterophagocytosis). LE activity in the plasma remained unchanged; hence CI produced no vital damage to lysosomes. Therefore, changes in the liver of rats kept under CI conditions are typical

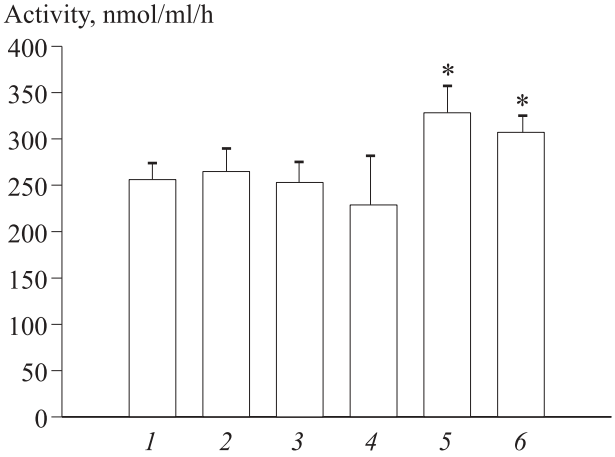

Fig. 2. Plasma β-galactosidase activity after administration of benz(a)pyrene and exposure to constant light.

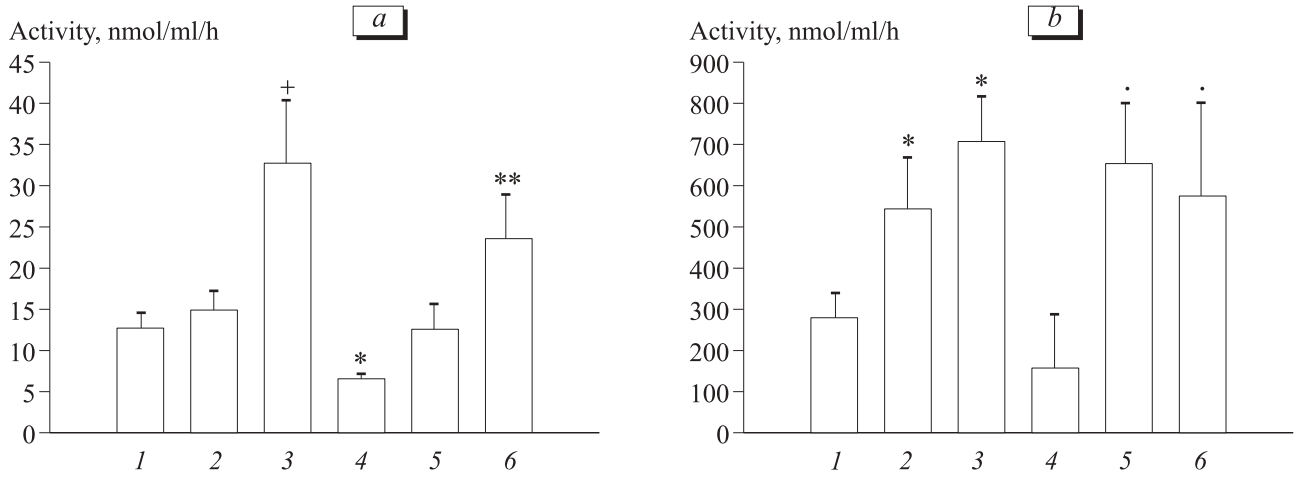

Fig. 3. Effect of benz(a)pyrene and constant light on biliary excretion of β-galactosidase (*a*) and N-acetyl-β,D-glucosaminidase (*b*). ***p*<0.05 compared to *5*.

of the stress-induced response in lysosomes [1]. BP (similarly to olive oil) did not potentiate the influence of CI on the stability of the lysosomal apparatus. Moreover, under these conditions BP decreased free LE activity to the control level. Probably, this compound prevented changes in the populational composition of lysosomes produced by CI.

Biliary excretion of LE depends on transport of lysosomes to the apical region of hepatocytes and exocytosis of the lysosomal content. BP stimulated biliary excretion of LE. Activities of β-galactosidase and N-acetyl-β,D-glucosaminidase in the bile increased by 2.5 times (Fig. 3). BP probably activates the synthesis and vesicular transport of protein from the Golgi apparatus to canalicular membranes. This process is typical of LE and various carrier proteins [11]. The increased secretion of LE in bile was not accompanied by a decrease in liver LE concentration. It can be suggested that enhanced release of LE is compensated by their synthesis in the endoplasmic reticulum and transport through the trans-Golgi network and apical region to the bile. Secretion of LE in the blood was not observed. Therefore, there is little likelihood that different pathways of LE transport are activated in liver cells. Published data show that 24-h excretion of LE reaches 3-5% of their content in the liver [2,12]. The 2.5-fold increase in the excretion of LE under the influence of BP did not rapid decreased their amount in the liver; the estimated 24-h reduction of enzyme activity was 10%. Therefore, considerable activation of enzyme biosynthesis was not required under these conditions.

CI exposure had an opposite effect and suppressed excretion of LE by 2 times. The stressogenic effect of CI probably inhibits physiological secretion of LE and release of the lysosomal content from the liver to the bile. These changes probably inhibit constitutive secretion of lysosomes and contribute to the syndrome of lysosomal accumulation.

CI tended to suppress BP-stimulated biliary excretion of the lysosomal content. CI exposure is followed by cell changes that probably have an adverse effect on the induction of LE excretion with the bile under the influence of BP. Physiological activation of the lysosomal apparatus under CI conditions prevents constitutive and BP-induced transport of LE to the bile. Administration of BP was more potent than CI in modulating the release of the lysosomal content to the bile. CI exposure in combination with BP administration stimulated the discharge of the lysosomal content and abolished functional activation of lysosomes.

Our results show that BP modulates vesicular transport of LE into the bile. These data indirectly indicates stimulation of vesicular transport from the trans-Golgi network to the apical region of hepatocytes. The process contributes to the transport of drug-carrying proteins to canalicular membranes [13]. It should be emphasized that carrier proteins are sometimes localized in lysosomal membranes [4]. Changes in activity of transport systems responsible for drug release into the bile should be evaluated in experiments with specific substrates of transmembrane transfer (doxorubicin and glutathione-S-methylfluorescein for mdr1 and mdr2 transporters, respectively).

REFERENCES

1. L. E. Panin and N. N. Mayanskaya, *Lysosomes: Role in Adaptation and Recovery* [in Russian], Novosibirsk (1987).
2. A. B. Pupyshev and V. I. Maiborodina, *Byull. Eksp. Biol. Med.*, **134**, No. 8, 163-168 (2002).
3. M. A. Tsartsidze, V. A. Lomsadze, and M. G. Shengeliya, *Vopr. Med. Khim.*, **30**, No. 1, 17-20 (1984).
4. L. Cabrita, T. C. Hobman, D. L. Hogue, *et al*., *Cancer Res*., **59**, 4890-4897 (1999).

5. M. Carevic and O. Carevic, *Toxicol. Lett*., **10**, No. 1, 41-49 (1982).
6. J. K. Chipman, J. K. Frost, P. C. Hirom, and P. Millburn, *Carcinogenesis*, **2**, No. 8, 741-745 (1981).
7. P. Deprez-Blummer, F. Levi, G. Metzger, and Y. Touitou, *Am. J. Physiol.*, **268**, No. 5, Pt. 2, R1111-R1116 (1995).
8. G. Goi, A. Fabi, A. Lombardo, L. Caimi, *et al*., *Chronobiologia*, **16**, No. 2, 93-103 (1989).
9. O. Hanson-Painton, M. J. Griffin, and J. Tang, *Biochem. Biophys. Res. Commun.*, **101**, No. 4, 1364-1371 (1981).
10. J. H. Hayes, C. J. Soroka, L. Rios-Velez, and J. L. Boyer, *Hepatology*, **29**, No. 2, 483-493 (1999).
11. M. Muller and P. L. M. Jansen, *Am. J. Physiol*., **272**, No. 35, G1285-G1303 (1997).
12. A. Nakano, D. L. Marks, P. S. Tietz, *et al*., *Hepatology*, **22**, No. 1, 262-266 (1995).
13. L. Payen, A. Courtois, S. Langonet, *et al*., *Toxicology*, **156**, Nos. 2-3, 109-117 (2001).
14. X.-H. Pei, Y. Nakanishi, K. Tokayama, *et al*., *J. Biol. Chem*., **274**, No. 49, 35, 240-35, 246 (1999).