EFFECT OF CHORIONIC GONADOTROPHIN ON PROSTAGLANDIN (E + A) LEVEL IN LIVER TISSUE AND BLOOD PLASMA OF RATS WITH CHRONIC HEPATITIS

I. M. Solopaeva, N. M. Roshchina, and N. L. Ivanova

UDC 616.36-008:94:577.175.859/-02:615.357.013.84

KEY WORDS: chorionic gonadotrophin; prostaglandins; hepatitis; liver; blood plasma.

The ability of chorionic gonadotrophin (CG) to act outside the gonads, specifically to stimulate regeneration of the normal and pathologically changed liver, was discovered previously and the suggestion was made that this hormone be used for the treatment of chronic hepatitis [10]. Normalization of the structure of the liver during stimulation of regeneration by CG in this disease is largely due to an increase in the number of normal and a decrease in the number of degenerating hepatocytes in a test area of the liver, and also to stimulation of intracellular generation in these cells [4, 5, 10, 11]. Under the influence of CG, protein synthesis in the liver has been shown to be intensified [6, 10], and in chronic disease the content of collagen [7] and neutral fat [12] in the liver is reduced, the phospholipid content is increased, and their fractional composition restored to normal [13]. All these observations suggest that CG is an effective stimulator of regeneration of the normal and pathologically changed liver.

CG has been shown to differ in its effect on different stages of immunogenesis. It stimulates migration of polypotent stem cells from the bone marrow [3] and of B and T lymphocytes from the thymus, but inhibits antibody formation [2]. These processes play an important role in the mechanism of stimulation of regeneration, for small lymphocytes are known to have morphogenetic properties and to transmit the proliferative stimulus [1], so that this hormone can be classed as a regulator of activity of the immune system, and one that is particularly valuable in autoimmune pathology.

Meanwhile the very important role of prostaglandins (PG) in the regulation of fundamental processes of vital activity of the cell under both normal and pathological conditions is known. Investigators are unanimous that the use of PG and their derivatives make pharmacotherapy more effective [9], more especially because patients with cirrhosis of the liver have been found to have hypoprostaglandinemia [8] and the view is held that PG may be possible initiators of regeneration of the liver after resection [15].

The object of this investigation was to study the effect of CG on the PG content in the liver and blood plasma in hepatitis.

EXPERIMENTAL METHOD

Experiments were carried out on 48 noninbred male albino rats weighing 180-240 g. Chronic hepatitis was induced by 20 subcutaneous injections of 0.3 ml of a 65% solution of carbon tetrachloride (CCl_4) in vegetable oil per animal on alternate days. As regards morphological changes and results of morphometric analysis, changes in the structure of the liver parenchyma of the animals were close to those described in the liver of children with chronic persistent hepatitis. A Soviet preparation of CG was used. It was injected in a dose of 150 U per animal 24 h after the last injection of CCl_4, on two successive days between 8 and 8.30 a.m. The rats were killed 24 and 48 h after injection of the hormone.

Clinical Morphological Laboratory, Gor'kii Research Institute of Pediatrics, Ministry of Health of the RSFSR. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 12, pp. 40-42, December, 1983. Original article submitted December 29, 1982.

 0007-4888/83/9612-1696$07.50

TABLE 1. Concentration of PG (E + A) in Liver Tissue (in ng/g) and Blood Plasma (in ng/ml) of rats (M ± m)

Group of animals	Time of investigation after beginning of injection of CG, h		
	24		48
	liver tissue	plasma	liver tissue
Intact rats	15,1±1,3	2,2±0,4	—
Rats with hepatitis receiving CG	43,1±4,0	4,6±0,7	79,0±2,8
not receiving CG	29,9±1,5*	2,3±0,3*	50,6±2,2*
Castrated rats with hepatitis: receiving CG	35,8±7,6	4,2±0,4	—
not receiving CG	10,0±1,1*	1,4±0,5*	—

Legend. *) Difference between PG (E + A) concentrations in rats receiving and not receiving CG is significant.

CG is known to stimulate PG synthesis in the testis [14]; some experiments were therefore conducted on animals after orchidectomy.

PG (E + A) were determined in liver tissue and blood plasma by radioimmunoassay using kits from Clinical Assays Inc., USA. A weighed sample of 500 mg tissue was used for analysis and homogenized with 1 ml phosphate buffer, pH 7.4, and 3 ml of a mixture of ethyl acetate with isopropanol and 0.2N HCl (3:3:1), 2 ml of ethyl acetate and 3 ml of distilled water were added to the homogenate, and the phases were separated by centrifugation. The organic phase was transferred to polypropylene tubes and dried at 55°C in a water bath.

To determine PG (E + A) 1 ml of blood plasma was mixed with 3 ml of petroleum benzene. After removal of the benzene phase, extraction of PG (E + A) was carried out in the same way as their extraction from tissue.

Radioactivity was counted in an SBS-1 scintillation counter.

EXPERIMENTAL RESULTS

Analysis of the results showed that the PG (E + A) content in the liver tissue of animals with hepatitis was twice as high as in intact rats (Table 1). This is in agreement with data [6] showing an increase in the total protein content in the liver tissue of these animals (369.4 mg/g compared with 314.7 mg/g in intact rats). Both these facts can be explained by the development of compensatory processes affecting the residual hepatocytes in response to injury and death of a large proportion of them during the development of experimental hepatitis. After injections of CCl_4 had been discontinued 43.7% of degenerating hepatocytes were found in the liver (compared with the normal 11.3%), despite the fact that the mean daily mitotic activity of the hepatocytes in the 2 days after discontinuing CCl_4 was higher (2.21 ± 0.30%) in these animals than in intact rats (0.23 ± 0.01%).

Cells with characteristic changes in their nuclei (karyopycnosis, karyorrhexis, karyolysis) and cytoplasm (hydropic and balloon degeneration), and also cells without nuclei were regarded as damaged or dying hepatocytes, conventionally described as degenerating. The total number of cells with the different signs of pathobiosis gave some idea of the total number of degenerating hepatocytes.

The PG (E + A) content in the liver tissue 24 h after injection of CG was increased by 1.4 times, and 48 h after the injection by 1.5 times compared with their concentration in the liver of untreated animals with toxic hepatitis at the same times of observation, and this was accompanied by a significant increase in the number of normal (6.7 ± 1.2% compared with 4.0 ± 0.6% before injection of CG, and with a normal level of 8.5 ± 0.2%) and a decrease in the number of degenerating (22.5%) hepatocytes. Meanwhile the total protein concentration

in the liver tissue remained high (337.8 mg/g) and the content of neutral fat in the hepatocytes was reduced (11.9% compared with 28% before injection of CG and with a normal level of 0-0.05%).

Hence, 24 and 48 h after injection of CG considerable stimulation of PG (E + A) synthesis was found in the pathologically changed liver, and this process plays an important role in the course of regenerative processes and normalization of the structure of the organ.

To obtain a more demonstrative proof of the ability of CG to stimulate PG (E + A) synthesis an experiment was carried out on male rats subjected to bilateral orchidectomy 1 day after the end of CCl_4 administration, the aim of the latter being to produce chronic hepatitis in the animals in accordance with the scheme described above.

It was found that 24 h after castration the PG (E + A) concentration in the rats' liver was reduced a little, but after injection of the hormone it was more than doubled compared with that in the intact rats, and became 3.5 times higher than in castrated untreated animals with toxic hepatitis at the same time.

These data confirm the stimulation of PG (E + A) synthesis in the tissue of the pathologically changed liver under the influence of CG, independently of their synthesis in the testis.

The PG (E + A) concentration was the same in blood plasma from intact rats and rats with chronic hepatitis. Injection of CG caused the PG (E + A) concentration in the blood plasma of the experimental animals to double. In castrated rats with toxic hepatitis the plasma PG (E + A) level was lower than in intact rats, but after injection of CG it increased threefold and became equal to the concentration of these substances in animals with intact testes. Comparison of the PG (E + A) concentrations in liver tissue and blood plasma suggests that the plasma PG (E + A) level largely depends on their synthesis in the liver.

Thus 24 and 48 h after stimulation of regeneration of the pathologically changed liver by CG, a marked increase in PG (E + A) synthesis was found in that organ, and this was connected to some degree also with an increase in the PG (E + A) concentration in the blood plasma.

The results suggest that during stimulation of repair processes in the pathologically changed liver PG (E + A) play an important role in the regulation and realization of the regenerative process. The results are not only important theoretically, since they explain some aspects of the mechanism of stimulation of regeneration of the liver by CG, but they are also of great practical value, for the method of treatment of chronic hepatitis with this hormone, suggested on the basis of the experimental data, can be regarded as prostaglandin therapy.

LITERATURE CITED

1. A. G. Babaeva, in: Physiology and Pathology of Connective Tissue [in Russian], Vol. 1, Novosibirsk (1980), pp. 4-5.
2. N. I. Gerasimova, in: Pharmacological Regulation of Regenerative Processes in Experimental and Clinical Practice [in Russian], Ioshkar-Ola (1979), pp. 136-139.
3. N. I. Gerasimova, I. F. Kolpashchikova, and I. M. Solopaeva, Author's Certificate No. 799764 (USSR).
4. T. F. Zhdanova, "Hepatocyte ultrastructure in chronic hepatitis and stimulation of regeneration by chorionic gonadotrophin," Author's Abstract of Candidate's Dissertation, Moscow (1980).
5. T. F. Zhdanova, in: Diseases of the Digestive Organs [in Russian], Moscow (1980), No. 12 (3).
6. N. L. Ivanova, in: Pharmacological Regulation of Regenerative Processes in Experimental and Clinical Practice [in Russian], Ioshkar-Ola (1979), pp. 139-142.
7. A. A. Kosykh, "Effect of different methods of stimulating regeneration of the cirrhotically stained rat liver on resorption of fibrosis," Candidate's Dissertation, Gor'kii (1974).
8. A. S. Loginov, M. N. Markova, N. V. Fedorova, et al., in: Current Problems in Gastroenterology [in Russian], No. 12, Moscow (1980), pp. 65-76.
9. I. S. Azhgikhina (ed.), Prostaglandins [in Russian], Moscow (1978).
10. I. M. Solopaeva, "Stimulation of regeneration of the normal and pathologically changed liver," Doctoral Dissertation, Gor'kii (1969).

11. I. M. Solopaeva, A. A. Kosykh, A. A. Volkova, et al., in: Current Problems in Gastroenterology [in Russian], No. 7, Moscow (1974), pp. 48-55.
12. I. M. Solopaeva, S. B. Stefanov, N. L. Ivanova, et al., in: Problems in Practical Gastroenterology [in Russian], Kaliningrad and Moscow (1979), pp. 106-107.
13. L. A. Tikhovodova, in: Proceedings of the 3rd Russian Republican Scientific and Practical Conference on Thermal Injuries [in Russian], Leningrad (1977), pp. 93-96.
14. F. Haour, B. Kouznetzova, F. Dray, et al., Life Sci., 24, 2151 (1979).
15. Y. Miura and N. Fukui, Cell. Mol. Biol., 25, 179 (1979).

ROLE OF LIPID PEROXIDATION IN INHIBITION OF CARDIAC Na,K-ATP-ase DURING STRESS

F. Z. Meerson, T. G. Sazontova,
V. E. Kagan, V. P. Tverdokhlib,
and Yu. V. Arkhipenko

UDC 616.127-008.939.15+616.7.127-008.931.5.577.152.361/-02:613.863

KEY WORDS: emotional-painful stress; lipid peroxidation; Na,K-ATP-ase.

During emotional-painful stress (EPS) lipid peroxidation (LPO) is activated [3] and Na,K-ATPase activity in the heart muscle is depressed [4]. This last effect can be prevented by injecting the β-blocker inderal before exposure to stress [4]. On the basis of these findings it can be posulated that depression of Na,K-ATPase activity in EPS is due to the fact that catecholamines activate LPO [2], with the result that the sarcolemma, the site of Na,K-ATPase, is damaged.

To test this hypothesis, in the first stage of the investigation described below the effect of LPO, induced in rats with EPS *in vivo* was evaluated, and in the second stage the effect of induction of LPO by an Fe^{++} + ascorbate system in the membrane fraction of the heart, rich in sarcolemma, was studied *in vitro*. In both stages the possibility of preventing the inactivating effect of LPO on Na,K-ATPase by 4-methyl-2,6-di-*tert*-butylphenol (ionol), an inhibitor of free-radical oxidation, was studied.

EXPERIMENTAL METHOD

Male Wistar rats weighing 180-200 g were used. Animals used in the first stage of the experiment were divided into four groups (8 rats in each group): 1) control, 2) EPS, 3) injection of the antioxidant ionol, 4) ionol followed by EPS. Ionol was injected intraperitoneally as a suspension in Tween-60 daily for 3 days before the experiment in a dose of 20 mg/kg body weight. EPS was induced in the form of an anxiety neurosis by the method in [8] in the course of 6 h. The animals were decapitated 2 h after the end of exposure to stress. As a result of EPS, all rats developed gastric ulcers.

The hearts were removed, freed from blood in ice-cold water, suspended, and frozen in liquid nitrogen. To isolate the membrane fraction the heart was homogenized for 30 sec without freezing in medium containing 10 mM imidazole and 1 mM EDTA, pH 7.5, in a homogenizer of "Politron" type, with the ratio of weight of tissue to volume of solution equal to 1:20. The resulting homogenate was passed through two layers of gauze and centrifuged for 20 min at 1000g. The residue was put through a Teflon-glass homogenizer and centrifuged under the same conditions. The last operation was repeated again. The residue was resuspended in the

Laboratory of Pathophysiology of the Heart, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Laboratory of Physical Chemistry of Biomembranes, M. V. Lomonosov Moscow University. (Planned by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 12, pp. 42-44, December, 1983. Original article submitted April 1, 1983.

