MORPHOLOGIC EFFECTS OF PHENOBARBITAL AND ZIXORIN ON RAT LIVER CELLS

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Treatment measures in liver damage are aimed primarily at the preservation of its metabolic and, in particular, its "detoxicating" functions, and also at stimulation of repair processes. The search for preparations activating the monooxygenase system of the liver, and also stimulating plastic processes in hepatocytes, is therefore an urgent problem. Preparations of this kind include phenobarbital sodium (Pb), an inducer of the mixed function oxygenase system (MFOS) of the liver, but its clinical use is restricted because of its side effects on the central nervous system [2-4].

The aim of this investigation was to study the possibility of using the Hungarian preparation zixoryn (Z),* another inducer of the liver MFOS, as a stimulator of plastic processes in hepatocytes, and using Pb as the comparison preparation.

EXPERIMENTAL METHOD

Male Wistar rats weighing 200-250 g, kept on the standard laboratory diet, were used. The animals were divided into three groups (five rats in each group). Rats of group 1 served as the control. To obtain the maximal inducing effect, animals of group 2 were given an intraperitoneal injection of Pb in a dose 50 mg/kg once daily for 4 days [5], and animals of group 3 were given Z by the intragastric route in a dose of 80 mg/kg, in Tween-80 (20% solution) once a day for 2 days [2]. The rats were decapitated under superficial ether anesthesia; animals of groups 2 and 3 were decapitated 24 h after the last dose of the preparation. Samples of liver for electron microscopy were fixed in 1% OSO_4 solution in phosphate buffer, pH 7.4, and embedded in Epon. Sections 1 μ thick were cut from the resulting blocks, stained with toluidine blue, and used for morphometry. Ultrathin sections were studied in the IEM-100S electron microscope. The cytoplasm of the hepatocytes from each animal at 20 sites was photographed with a magnification of 7000. The negatives thus obtained were subjected to stereometry, using closed test systems of squares [10]. The differences between the mean values compared were taken to be significant at the $p < 0.05$ level.

EXPERIMENTAL RESULTS

The effect of induction in response to Pb and Z at the cellular level was manifested as an increase in volume of the hepatocytes due to an increase in volumes of both cytoplasm and nuclei (Fig. 1). Injection of Pb caused an increase in the volumes of hepatocytes, cytoplasm, and nuclei by 74, 77, and 42.7% respectively, and injection of Z led to increases of 32, 33, and 23.7% compared with the control.

*Alt. name flumecinol.

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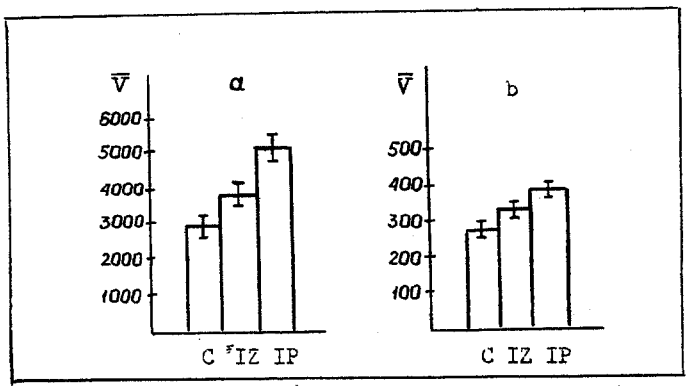

Fig. 1. Results of morphometry of hepatocyte cytoplasm and nuclei: $\bar{V}$) volume of cytoplasmic structures, in μ^3; *a*) volume of cytoplasm of hepatocytes, b) volume of nuclei of hepatocytes; C) control; IZ) induction by zixoryn, IP) induction by phenobarbital.

TABLE 1. Results of Morphometry of Hepatocyte Ultrastructures after Injection of Phenobarbital and Zixoryn (M ± m)

Parameters tested	Group of animals		
	control	induction by phenobarbital	induction by zixorin
Mitochondria, V_V	31,3±0,95	23,99±1,08*	28,73±0,94
outer membrane (S_V)	1,54±0,04	1,14±0,04*	1,42±0,04
inner membrane (S_V)	7,19±0,30	5,34±0,33*	7,58±0,42
Peroxisomes (V_V)	1,40±0,12	1,10±0,12	1,46±0,13
Lysosomes (V_V)	0,48±0,09	0,45±0,08	0,54±0,09
Smooth endoplasmic reticulum: V_V	3,80±0,47	11,54±1,13*	9,13±0,86*
S_V	1,66±0,14	3,81±0,33*	2,73±0,22*
Rough endoplasmic reticulum: V_V	12,4±0,90	13,47±1,72	16,4±1,04*
S_V	4,49±0,20	3,57±0,26*	5,01±0.25
Ribosomes: pre N_A	1,85±0,65	1,17±0,60	1,02±0,50
attached (N_A)	0,41±0,15	0,17±0,08	0,22±0,10
	26,85±1,9	22,7±1,9	23,68±1,75
	36,6±2,8	28,02±1,9*	38,52±2,53

Legend: V_v) Bulk density of structures (in % of volume of cytoplasm), S_v) surface density of structures (in μ^2/μ^3 volume of cytoplasm), N_A) number of ribosomes in 1 μ^2 area of section through cytoplasm, here and in Table 2: *) significant difference from control.

Investigation of hepatocytes at the subcellular level showed that the increase in their volumes was due to hypertrophy. We know that induction of liver MFOS is brought about by an increase in the quantity of enzymes and membranes of the smooth endoplasmic reticulum (SER) [1, 4, 6-8]. The stereometric data are in agreement with observations in the literature indicating that the dominant morphologic criterion of activation of MFOS at the subcellular level is hyperplasia of the SER (Table 1). For instance, on induction by Pb, the bulk and surface densities of SER were increased by 3 and 2.6 times, whereas induction by Z they were increased by 2.4 times and 64.6% respectively compared with the control. However, on induction with Pb, hyperplasia of SER was evidently connected with redistribution of plastic material in its favor, for the total bulk and surface densities of the cytoplasmic organoids (without the SER and Golgi complex – GC) were reduced by 14.4 and 24% respectively compared with the control. However, these same parameters, calculated for organoids, did not differ significantly from the control (Table 2). This redistribution also took place, evidently, with a decrease in the energy-forming function of the hepatocytes, as shown by a decrease of 23.4% in the bulk density of the mitochondria and of 25.6 and 25.7% respectively of the surface densities of their outer and inner membranes. Decreases in surface density of the rough endoplasmic reticulum by 20.5% and in the number of attached ribosomes by 23% compared with the control also indicate depression of the external secretory function of the hepatocytes as a result of injection of Pb.

TABLE 2. Results of Morphometry of Surface and Bulk Densities of Cytoplasmic Structures of Hepatocytes after Injection of Phenobarbital and Zixoryn (M ± m)

Parameters tested	Group of animals		
	control	induction by phenobarbital	induction by zixorin
ΣS_v —Total cytoplasmic organoids	15,29±0,42	14,04±0,54	16,96±0,55*
ΣS_v —Cytoplasmic organoids without SER and GC	13,22±0,36	10,05±0,42*	14,01±0,49
ΣV_v — Total cytoplasmic organoids	51,23±1,54	51,72±2,12	57,28±1,73*
ΣV_v — Cytoplasmic organoids without SER and GC	45,58±1,30	39,01±1,70*	48,15±1,41

Legend. ΣS_v) Total surface density of membranes of organoids (in μ^2/μ^3 cytoplasm of hepatocytes), ΣV_v) total bulk density of organoids (in %).

On induction by Z, besides hyperplasia of SER, there was also an increase in the volume of cytoplasm proportional to hyperplasia of all the cytoplasmic organoids. For instance, the total bulk and surface densities of the organoids (without SER or GC) did not differ significantly from the control, but these same parameters, calculated for all organoids including SER and GC increased by 11.8 and 10.9% respectively.

It thus follows from the results described above that zixoryn can be used to stimulate repair processes in the liver, and from this point of view it is preferable to Pb, because it has greater ability to induce stimulation of plastic processes in hepatocytes and it is free from the side effects of Pb.

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