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SPATIOTEMPORAL CHARACTERISTICS OF BETA-HYDROXYBUTYRATE DEHYDROGENASE ACTIVITY IN THE RAT LIVER LOBULE

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KEY WORDS: beta-hydroxybutyrate dehydrogenase, spatiotemporal organization, metabolic processes.

The spatiotemporal organization of metabolism in the liver lobule is discussed in reports of investigations into structural and functional heterogeneity of hepatocytes and biological rhythms of their functions [2-4, 7, 8]. Data are given in the literature on the spatial distribution of enzymes in the liver lobule and changes in their activity during the 24-h period [6, 10]. Meanwhile there have been no investigations in which a spatial and temporal approach has been made to study the structural and functional parameters in the liver lobule.

The object of the present investigation was to make a parallel study of the spatial and temporal principles governing activity of beta-hydroxybutyrate dehydrogenase (BDH) in the liver lobule of normal rats.

EXPERIMENTAL METHOD

Experiments were carried out on 30 noninbred male albino rats weighing 180-200 g. For 2 weeks before sacrifice the animals were kept under conditions of 12 h daylight (9 a.m. to 9 p.m.) and 12 h of darkness; the intensity of illumination was 300 lx. The rats were decapitated at 1, 5, and 9 a.m. and 1, 5, and 9 p.m. Five animals were killed at each time. BDH activity in the hepatocytes was determined histochemically by the method of Nachlas et al. [5] in 12-μ frozen sections. Activity of the enzyme (EA) was determined quantitatively in five liver lobules of the same size from each animal on a modified scanning integrating digital microspectrophotometer [1], using a 10 × 10 frame with probe 0.5μ in diameter, in a monochromatic beam with wavelength 560 nm, and with a linear stepwise scanning time for the whole frame of 1-2 sec. EA was expressed in conventional units of total optical density of the test object. Lobules in which 18 cells were arranged along a line joining the central vein and the portal vessels (radius of the lobule) were analyzed. Cells along 2-4 radii were examined photometrically in each lobule. The radius was divided into three equal parts, with six cells in each part. These parts corresponded to circulatory zones 1, 2, and 3 of the hepatic acinus as described by Rappaport [9], in the direction from the periphery to the center of the lobule. Each of the above zones was subdivided into two equal subzones (three cells along the radius). The mean value of EA was calculated in the zones and subzones of the lobule, and also in cells occupying the same position on the radius. To describe changes in EA from cell to cell, the difference between the EA level in two neighboring hepatocytes was determined along the whole length of the radius of the lobule. This parameter was used to calculate the mean value of the change in EA for the same cell position in the whole lobule and its zones. The different parameters characterizing EA and its changes in the lobule were determined at each period of the experiment and on average for the 24-h period. The numerical results were subjected to statistical analysis by the Fisher–Student method, at the $P \leqslant 0.05$ level of significance.

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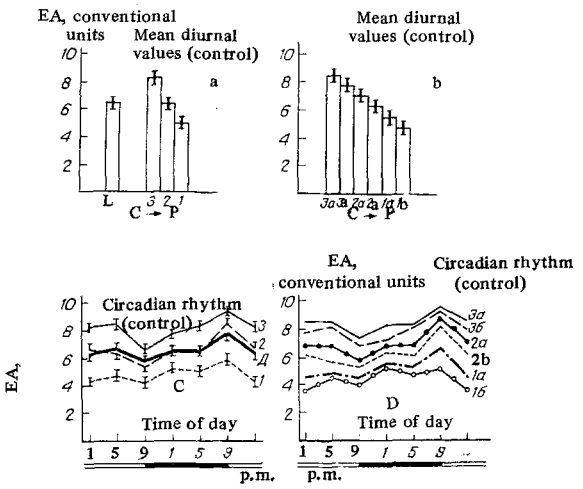

Fig. 1

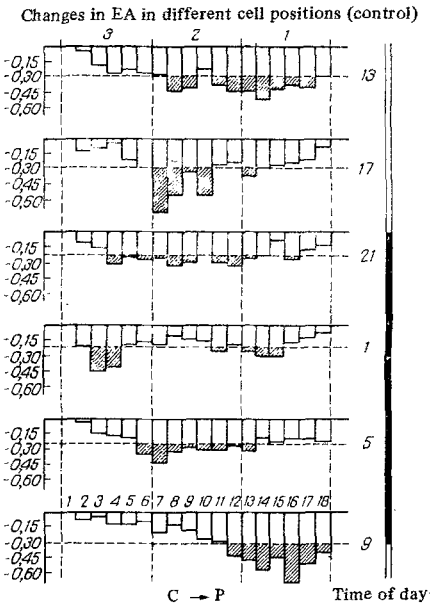

Fig. 2

Fig. 1. Spatial and temporal dynamics of BDH activity in liver lobule of normal rats: a) mean diurnal BDH activity in whole lobule (L) and its zones 3, 2, and 1; b) mean diurnal values of BDH activity in subzones of lobule; c, d) change in BDH activity in whole lobule (L) and its zones and subzones during 24-h period.

Fig. 2. Relative changes in BDH activity in different cell positions in liver lobule of normal rats during 24-h period. 1-3 Above) zones; 1-18 below) cell positions in lobule.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 (c, d) that a monophasic circadian rhythm of EA exists in the whole lobule and its zone and subzones, with a maximum at 9 a.m. and a minimum at 9 p.m. ($P = 0.001$-0.014). The relative amplitude of the rhythms was highest (1.53 and 1.50) in subzones 3a and 1b. Synchronization of the rhythmic changes in EA was more marked in subzones 3a, 3b, 2a, and 2b. The increase in EA in this rhythm in subzones 1a and 1b was observed earlier (a shift to the left by 5.6 h). In the first four subzones of the lobule from center to periphery the active phase of EA rhythms was recorded at the end of darkness and at the beginning of the daylight period of the 24 hours, but in the two peripheral subzones the active phases were located in the period of darkness.

According to the mean diurnal values (Fig. 1a, b) the highest EA was observed in the central parts of the lobule, and it increased gradually toward the periphery. The degree of decrease in EA was greater at the periphery of the lobule than at its center. For instance, differences in the mean diurnal values of EA between neighboring subzones were 6-10% in zones 3 and 2, but 14-15% in zone 1.

Diurnal fluctuations in the mean value of the changes in EA for the same cell position were found in the whole lobule and its zones. In the lobule the changes reached a maximum during the period of daylight from 9 a.m. to 5 p.m. and a minimum at 9 p.m. Rhythmic fluctuations in this value were not synchronous in individual zones of the lobule. Whereas it was maximal in zone 3 at 1 a.m., in zone 2 this happened at 5 p.m. and in zone 1 at 9 a.m.

The mean diurnal value of the changes in EA in the same cell position was lowest in zone 3. It was higher in zones 2 and 1 than in zone 3, by 69 and 75% respectively ($P = 0.037$-0.050).

The maximal value of the relative change in EA in corresponding cell positions (Fig. 2) was located in different parts of the lobule depending on the time of day. For instance, at 1 a.m. the greatest changes in this value were observed in zone 3. At the next time of investigation (5 a.m.) they were found in zone 2, later (9 a.m.) in zone 1, next (1 p.m.) they gradually moved into zone 2, in which they reached a maximum at 5 p.m., and at 9 p.m. they were again observed in zone 3. The maximal value of the changes in EA from cell to cell thus shows a circadian rhythm of spatial displacement over the zones of the lobule.

These results are evidence that the level of BDH activity in rat hepatocytes depends on their spatial arrangement in the lobule and the time of day. The existence of a circadian rhythm of spatial displacement of the maximal value of

differences in EA between neighboring hepatocytes over the zones of the lobule points to the absence of any constant spatial localization of hepatocyte populations, differing from each other the most in their EA, in the lobule. Consequently, if functional heterogeneity of hepatocytes (with respect to EA, for example) does exist it is dynamic in character both in space and in time.

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POSSIBILITY OF FORMATION OF BRUSH CELLS FROM TYPE II ALVEOLOCYTES IN RATS

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KEY WORDS: type II alveolycyte; brush alveolocyte; alveolocyte of intermediate type; differentiation.

Besides flat respiratory (type I) alveolocytes and the large type (II) which secrete lung surfactants, at present another third type of these cells is distinguished in the composition of the alveolar epithelium of many laboratory animals, namely "brush" alveolocytes (BA) [1, 5, 10, 14]. The ultrastructure of BA has been studied in detail by both transmission [1, 5, 10, 12, 13] and scanning [1, 9] electron microscopy, whereas the origin of these cells and the character of their interaction with other pneumocytes, including alveolocytes, has not been established.

In this investigation an attempt was made to study the character of intercellular interactions between alveolocytes in the solitary lung remaining in rats after left-sided pneumonectomy, in order to determine whether type II alveolocytes can differentiate into BA, the number of which in the alveoli rises sharply toward the end of the first week after the operation [6].

EXPERIMENTAL METHOD

The lungs of male rats weighing 140-200 g, some intact and others 5-7 days after left-sided pneumonectomy, were fixed by perfusion with 3.6% glutaraldehyde solution in 0.1M cacodylate buffer (pH 7.4) through the pulmonary artery followed by postfixation of pieces of tissue in 1% OsO_4 solution. Material for electron microscopy was processed by the usual method. Semithin sections 2μ thick, stained metachromatically with toluidine blue, were used for preliminary detection of BA under the light microscope and for trimming the blocks [5]. Ultrathin sections were examined in the JEM-100B electron microscope.

EXPERIMENTAL RESULTS

BA of the normal and hypertrophied rat lung were found most frequently in the region of alveolar nodes (junctions of 2-3 alveoli) or in thickened areas of the alveolar walls. The cells lay on a basement membrane which was common for all

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