Since the orienting reflex is characterized by nonspecificity of the reflexogenic zone and by a low threshold of sensitivity [3, 9], the great majority of neurons of the snail is involved in the reaction and their excitability is enhanced; it is probably this which leads to such a sharp increase in the content of this protein in the whole of the animal's CNS, both in the early stages of learning and on arousal. During further learning the raised protein level persists only in a limited number of neurons directly concerned with the reflex formed [4, 11]. It can thus be postulated that this effect of an increase in content of acid protein is connected with the orienting reflex and virtually disappears when that reflex is extinguished.

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CHANGES IN DISTRIBUTION OF ISOCITRATE DEHYDROGENASE ACTIVITY WITH TIME IN RAT LIVER

V. V. Markina

UDC 621.351.11"52"

KEY WORDS: liver; isocitrate dehydrogenases; enzyme activity

Enzyme activity (EA) in hepatocytes fluctuates in the 24-h period and differs in different parts of the hepatic lobule [1, 4-7]. However, correlation between the topographic distribution of enzymes in the hepatic lobule and circadium rhythms of their activity has virtually never been studied. The presence of such correlation was established previously as changes in the distribution of activity of β-hydroxybutyrate dehydrogenase activity with time in rat hepatocytes [1]. The aim of this investigation was to determine the character of changes in the distribution of activity of NAD- and NADP-dependent isocitrate dehydrogenase (IC-NAD and IC-NADP respectively) in the hepatic lobule or normal rats with time.

EXPERIMENTAL METHOD

Experiments were carried out on 30 noninbred albino rats weighing 180-200 g. Before sacrifice the animals were kept for 2 weeks in artificial light with alternation of 12 h light (from 9 a.m. to 9 p.m., intensity of illumination 300 lx) and 12 h darkness. The rats were decapitated at 9 a.m., 1, 5, and 9 p.m., and 1 and 5 a.m. At each experimental point 4 or 5 animals were used. IC-NAD IC-NADP activity in the hepatocytes was determined by the method in [3] in frozen sections 12 μ thick. The technique of quantitative determination and calculation of the parameters for subsequent analysis was described previously [1]. To record changes in the EA level among the cell positions graphically, differences in EA between neighboring cells were expressed as percentages, and changes exceeding the coefficient of variation of EA in the zone of the lobule at each stage of the experiment were taken to be

Department of Histology and Embryology, N. A. Semashko Moscow Medical Stomatologic Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. A. Minkh.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 98, No. 10, pp. 431-434, October, 1984. Original article submitted January 13, 1984.

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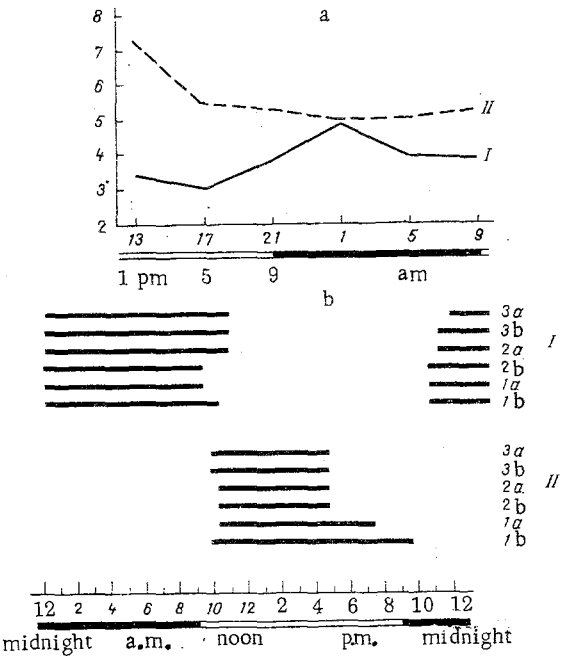

Fig. 1. Time changes in IC-NAD (I) and IC-NADP (II) activity in hepatic lobule of normal rats. Abscissa, clock time; ordinate, EA (conventional units). a) Circadian rhythms of EA in whole lobule; b) phase diagrams of circadian rhythms of EA in subzones of lobule (bold lines indicate active phases of rhythms).

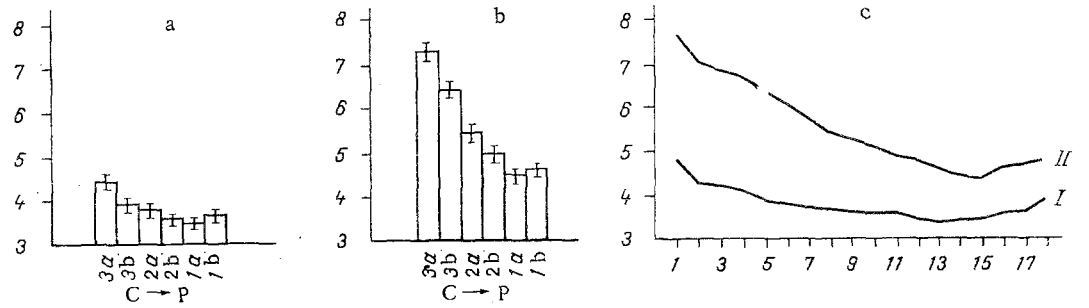

Fig. 2. Changes in distribution of IC-NAD (I) and IC-NADP (II) activity in hepatic lobule of normal rats. Ordinate: EA (in conventional units). a, b) Average values of activity for 24-h period of IC-NAD and IC-NADP respectively in subzones of lobule, c) changes in EA distributed among cell positions, C) center, P) periphery of lobule.

significant. To determine the parameters of the circadiam rhythm of EA and to construct phase diagrams of the rhythms, the method in [2] was used. The results were analyzed by the Fisher—Student test, with a 0.05 level of significance of differences. The coefficient of correlation (r) between changes in EA in hepatocytes in different subzones of the lobule in the course of the 24-h period also was determined.

EXPERIMENTAL RESULTS

A monophasic circadian rhythm of IC-NAD activity was found in the hepatic lobule with a peak at 1 a.m. and a trough at 5 p.m. ($P \leqslant 0.001$) (Fig. 1a). The relative amplitude of the rhythm was 1.58. Monophasic rhythms of EA were typical of hepatocytes in all subzones of the lobule, with acrophase at 1 a.m. and active phases between 9 p.m. and 10 a.m., i.e., during the dark period of the 24-h cycle (Fig. 1b). Evidence of the high degree of synchronization of rhythmic changes in IC-NAD activity in different subzones of the lobule independently of their distance apart was given by values of r, which were 0.82-0.92. The relative amplitude of the circadian variations in EA increased somewhat in the direction from center to periphery of the lobule (1.45 in subzone 3a, 1.76, and 1.72 in subzones 1a and 1b respectively). The length of the active phase of the EA rhythms was about equal in different subzones (noon to 1 p.m.).

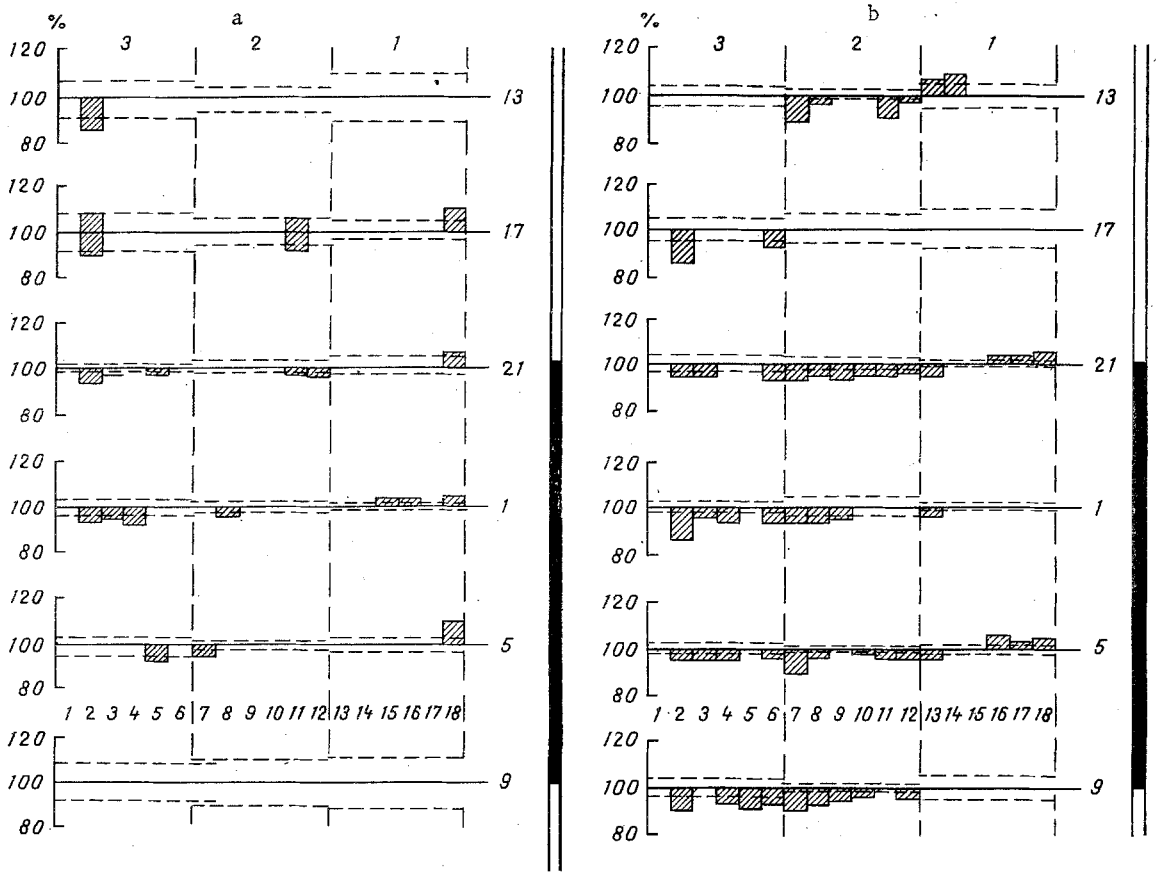

Fig. 3. Location of highest relative value of changes in IC-NAD (a) and IC-NADP (b) activity between neighboring cells in lobule during 24-h period. Nos. 1-3 on top represent zones, Nos. 1-18 below represent cell positions in lobule.

The highest mean value of IC-NAD activity for the 24-h period was observed in subzone 3a. In subzone 2a it was 16% lower ($P = 0.037$), and in subzones 2a and 1b it did not differ significantly (Fig. 2a). The decrease in EA from center toward periphery of the lobule (Fig. 2c) was observed as far as cell position 14 (by 28% compared with cell position 1, $P = 0.003$), and the day after as far as cell position 18 there was a small increase in EA (by 12%), although this was not significant ($P > 0.05$). Average changes in levels of IC-NAD activity for the 24-h period were most significant between cells in positions 1 and 2 and between cells in positions 17 and 18.

The main difference between levels of activity of IC-NAD between neighboring cells for the 24-h period was higher in zones 3 (−1.17) than in zone 2 (−0.07, $P = 0.005$), whereas in zone 1 the changes in EA were opposite in character (+0.06). Maximal changes in EA per cell position in the 24-h period were observed in zone 3 between 1 a.m. and 1 p.m., in zone 2 between 5 a.m. and 5 p.m., and in zone 1 between 9 a.m. and 1 p.m.

The highest relative change in IC-NAD activity from cell to cell showed a definite cycle of displacement over the zones of lobule during the 24-h period (Fig. 3). At 1 p.m. it was observed only in zone 3, between 5 p.m. and 5 a.m. it was recorded in all the zones, but at 9 a.m. these changes in EA were uniform throughout the lobule.

Determination of IC-NAD activity showed (Fig. 1a) a time course in the lobule reflected by a monophasic circadian rhythm with maximum at 1 p.m. and minimum at 1 a.m. ($P = 0.006$) and with a relative amplitude of 1.47. Monophasic rhythms of EA were observed in all subzones of the lobule, with acrophases at 1 p.m., and active phases beginning at 9.30-10 a.m.; however, whereas in subzones 3a and 2b they ended at 4.30 p.m., in subzones 1a and 1b they ended at 7 and 9 a.m. respectively (Fig. 1b). Rhythms of EA in the central and middle subzones of the lobule are thus characterized by a shorter duration of their active phase (6.5-7 h) than in peripheral subzones (9-11.5 h). For that reason, whereas the active phase in subzones 3a and 2b occupies the first half of the period of daylight, in subzones 1a and 1b it occupies practically the whole of the daylight period. The relative amplitude increased from subzone

2b (1.42 and 1.63), but then decreased (1.42 and 1.21 in subzones 1a and 1b respectively). Consequently, rhythmic changes in IC-NADP activity in the different subzones of the lobule are to some degree asynchronous. This is confirmed by the data of correlation analysis, which showed a decrease in r between circadian fluctuations of EA in the subzones with their increasing topographic distance apart. Between neighboring subzones r = 0.88, whereas between central and peripheral subzones r = 0.62.

The mean level of IC-NADP activity in the 24-h period was highest in subzone 3a (Fig. 2b). In the direction toward the periphery of the lobule it decreased, and in zone 2a it was 76% of its value in subzone 3a ($P = 0.019$). A decrease in EA was observed in cell position 16 (by 41% compared with cell position 1, $P < 0.001$), thereafter to cell position 18 it increased a little (by 11%), but these changes were not significant ($P > 0.05$, see Fig. 2c). Mean values of EA in subzones 2a and 1b for the 24-h period likewise did not differ significantly. On the other hand, IC-NAD showed the most marked fluctuations in IC-NADP activity in the 24-h period between neighboring cells in all zones of the lobule, whereas the increase in EA at its periphery were less marked.

The highest mean value for changes in IC-NADP activity per cell position for the 24-h period was observed in zone 3 (—0.30), it was lower in zone 2 (+0.23), and quite small in zone 1 (—0.01). Changes in EA from cell to cell were greatest in zone 3 from 5 to 9 p.m., and in zones 2 and 1 at 1 p.m.

The maximal relative value of changes in IC-NADP activity between neighboring cells was topographically inconstant in the lobule during the 24-h period (Fig. 3). Whereas at 1 p.m. it was observed in zone 2 and in subzones 3b and 1a, at 5 p.m. it was recorded in zone 3 only. Between 9 p.m. and 5 a.m. it was found in all zones, but at 9 a.m. only in zones 3 and 2.

These data are evidence that the level of activity of IC-NAD and IC-NADP in rat hepatocytes changes in the course of the 24-h period and differs in different parts of the hepatic lobule. Circadian rhythms of activity of these enzymes are out of phase: IC-NAD activity is increased in the dark period whereas IC-NADP activity is increased in the light period of the 24-h cycle. Rhythmic changes in IC-NADP activity in different subzones of the lobule during the 24-h period are less synchronized than circadian changes in IC-NAD activity. This indicates that the character of rhythms of IC-NADP activity in hepatocytes is more dependent on their topographic position in the lobule. Changes in distribution of activity of these two enzymes were largely similar (they decreased in the direction from center toward periphery of the lobule as far as positions 14-16, and then increased a little), although the mean range of these changes for the 24-h period between cell positions 1 and 18 was greater for IC-NADP than for IC-NAD (1.62 and 1.24 respectively). Moreover, whereas the mean changes in IC-NAD from cell to cell during the 24-h period were greatest in the center and at the periphery of the lobule, similar changes in IC-NADP activity were found in all zones of the lobule. The greatest value of differences in the activity of the two enzymes between neighboring hepatocytes showed a regular spatial distribution in the lobule during the 24-h cycle as was discovered previously for β-hydroxybutyrate hydrogenases [1]. The results of the present investigation indicate that functional heterogeneity of hepatocytes, especially as regards the level of EA, must be studied in close connection with the time course and topography of changes in metabolism of these cells.

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