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CIRCENNIAL RHYTHM OF MYOCARDIAL ARYL SULFATASE ACTIVITY IN INTACT RABBITS

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KEY WORDS: aryl sulfatase; lysosomes; heart

There is evidence [1, 4] that some parameters of cardiac activity follow a distinct circennial rhythm. It is interesting to examine the principles governing analogous changes in lysosomal activity of the cardiomyocytes, for these organelles can play an important role in intracellular regeneration processes [3] and, consequently, they can determine both metabolism of the myocardium and its contractile function.

The aim of this investigation was to examine the state of the lysosomal apparatus of the cardiomyocytes of intact rabbits in the course of the year by the use of an enzyme histochemical reaction for detection of aryl sulfatase activity, regarded [5] as a selective method of enzyme histochemical identification of lysosomes.

EXPERIMENTAL METHOD

Experiments were carried out on 36 male Chinchilla rabbits weighing 2.5-3 kg. Every month (on the 21st-23rd of each month) three rabbits were killed by thoracotomy under superficial hexobarbital anesthesia, after which the heart was extracted. Aryl sulfatase activity was determined histochemically [2] in frozen sections of the left and right ventricles. Activity of the enzyme was assessed by counting the number of dark brown granules in 30 fields of vision (for each rabbit) under immersion magnification. The number of fields of vision (out of a total of 90) in which granules were found also was counted. The numerical data were subjected to statistical analysis by Student's test using a "Commodore 64" personal computer. The difference between mean values was taken to be significant at the $p \leq 0.05$ ($T \geq 2.0$) level. Correlation between the parameters was assessed as strong if the absolute value of the coefficient of correlation $r \geq 0.7$, as moderately strong if $r = 0.69$-0.3, and as weak if $r = 0.29$. The significance of the correlations was estimated by the usual statistical methods, based on our own program for the personal computer.

EXPERIMENTAL RESULTS

The results are given in Table 1.

The results in Table 1 are evidence that activity of the lysosomal enzyme aryl sulfatase in both left and right ventricles has a distinct circennial rhythm. Differences between mean values for neighboring months are not significant for the left ventricle during the periods February—May and September—October, and for the right ventricle during the periods February—April and June—August. At all other times the difference between the mean values is significant. Over the whole period of the investigation, except in September, the difference between the mean values for the number of granules in the left and right ventricles is significant.

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TABLE 1. Circennial Rhythm of Aryl Sulfatase Activity in Rabbit Myocardium

Month	Left ventricle		Right ventricle	
	a	b	a	b
January	7,42±0,85	46	3,04±0,44	35
February	14,03±1,05	65	5,92±0,58	53
March	14,03±0,9	73	4,97±0,84	36
April	13,16±0,97	64	4,91±0,84	43
May	14,62±0,39	90	6,20±0,56	57
June	9,57±0,72	64	5,73±0,65	49
July	10,09±0,62	70	5,30±0,55	50
August	14,35±1,09	68	6,58±0,70	51
September	2,73±0,44	32	2,38±0,36	32
October	2,31±0,42	25	1,01±0,24	16
November	5,68±0,83	33	2,44±0,41	28
December	12,30±1,20	57	3,69±0,52	36

Legend. a) Number of granules in field of vision, b) number of fields of vision with granules.

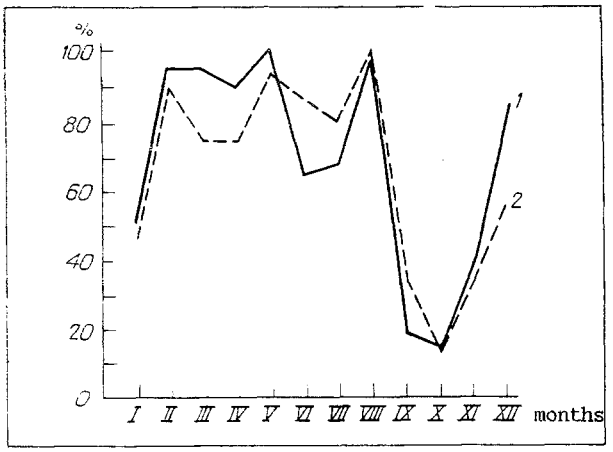

Fig. 1. Circennial rhythm of aryl sulfatase activity (mean number of granules per field of vision) in left (1) and right (2) ventricles.

Curves characterizing the circennial rhythm of the number of aryl sulfatase granules in the left and right ventricles are given in Fig. 1 in which, for the sake of clarity, they are shown as percentages. It follows from Fig. 1 that four significant increases were observed in the number of granules counted during the last month of each season in the two ventricles, and four significant decreases characteristic of the middle month of each season (except for the left ventricle in summer, where, when the fall took place not in July, but in June, and the difference between the mean values in this case, as was mentioned above, is not significant). On the whole, aryl sulfatase activity in the two ventricles was increased in the winter and spring, and at the end of summer, but in the fall it fell significantly.

The level of aryl sulfatase activity, as is clear from Table 1, was significantly lower in the right ventricle than in the left, but fluctuations of this parameter, expressed as percentages, were virtually identical for the two ventricles (Fig. 1).

Correlation analysis showed strong and significant positive correlation ($r = +0.88$) between the series of annual values of aryl sulfatase activity in the two ventricles.

As regards the number of fields of vision in which granules were discovered, in the left ventricle all fields of vision contained granules of the enzyme in May, but in October the degree of saturation of the cardiomyocytes with the enzyme was lowest (granules were found in 25 of 90 fields of vision). Strong and significant positive correlation also was found for this parameter between the left and right ventricles ($r = +0.87$).

Thus a distinct rhythm of lysosomal activity is observed in the myocardium of both ventricles of the heart, and its identical character in the left and right ventricles indicates that this process is controlled by the same regulatory influences, although the initial level of lysosomal activity is lower in the right ventricle than in the left.

It can be tentatively suggested that such marked differences in the monthly activity of the lysosomal apparatus must have a significant influence also on the level of physiological intracellular regeneration of the cardiomyocytes.

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LIMITING THE AREA OF ISCHEMIC NECROSIS BY THE ANTIOXIDANT IONOL

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KEY WORDS: ischemic necrosis; ionol; morphometry

The fact that the antioxidant ionol limits the area of the zone of necrosis in experimental myocardial infarction was first established comparatively recently [3] and direct confirmation has been obtained more recently [1]. However, these investigations with injection of ionol before or after the creation of an experimental infarct leave a number of questions unanswered. For instance, data on the pharmacokinetics of ionol show that only traces of the antioxidant remain in the body 24 h after its administration [9]. Consequently, the protective effect of ionol in the investigations cited above can hardly be dependent on its preventive action, but is realized mainly after coronary occlusion has taken place and an ischemic focus has been formed, i.e., when virtually no blood flow is present in the ischemic zone, into which significant quantities of antioxidants are unable to penetrate. It is thus not clear how in fact the zone of necrosis is reduced in size. Another very important factor is that the area of the zone of necrosis is usually measured by one of the existing methods: planimetrically or by manual or computerized morphometry.

The aim of this investigation was, first, to compare the action of ionol as an inhibitor of lipid peroxidation in and outside the zone of ischemia, and second, to assess the effect of the method used to determine the area of the zone of necrosis on the results of the investigation by using methods of manual and computerized morphometry simultaneously but independently.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 180-200 g. Experimental myocardial infarction was created by Selye's method by ligation of the descending branch of the left coronary artery. Animals with experimental myocardial infarction were divided into two groups. Ionol was given perorally in olive oil in a dose of 100 mg/kg daily for 3 days before creation of the infarct and 2 and 24 h thereafter. The hearts of animals receiving ionol and, at the same time, hearts of control animals not receiving the antioxidant, were taken 48 h after creation of the experimental myocardial infarct. Biochemical tests were carried out for diene conjugates [5], Schiff bases [6], and activity of antioxidative enzymes, namely glutathione peroxidase, as in [7], and catalase, as in [8]. Serial histotopographic sections through the heart, stained with nitro-BT for succinate dehydrogenase, were used to measure the area of the necrotic tissue. Sections were cut every 1.5 mm, starting from the apex of the heart and in the direction of its base.

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