INFLUENCE OF AMINOPTERIN ON COMPENSATORY CARDIAC HYPERTROPHY

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It has been shown [2, 3] that intensification of the contractile function of the heart causes activation of nucleic acid and protein synthesis in the myocardium, this being a manifestation of the influence of physiological functioning on the genetic apparatus of the cell and forming the basis for myocardial hypertrophy. It was important to determine to which portion of the genetic apparatus the activating influence of intensified physiological functioning is addressed.

It has been established [2] that in myocardial hyperfunctioning the DNA content of the myocardium rises in direct proportion to the increase in the mass of the heart. These data were later confirmed by researchers who discovered that the incorporation of tagged predecessors into DNA and RNA increases during the first stage of cardiac hyperfunctioning and hypertrophy. This means that DNA synthesis is activated under the influence of intensified physiological functioning. In this connection it was important to establish whether or not activation of DNA synthesis in the myocardial cells is necessary for activation of protein synthesis and the development of hypertrophy. F. Z. Meerson suggests the use of a broad spectrum of nucleic acid inhibitors and stimulators for solving problems of this type [3].

We investigated the influence of aminopterin, an agent which blocks DNA synthesis, on protein synthesis and the development of myocardial hypertrophy in compensatory cardiac hyperfunctioning.

EXPERIMENTAL METHOD

The experiments were conducted on 151 male white rats weighing 100-235 g. The animals were divided into four groups. The first, control group comprised 26 animals. The rats (22) in the second group received aminopterin. Aortic coarctation was created in the rats (36) of the 3rd group by Beznak's method [6] as modified by Kogan [1] and Pshennikova et al. [4]; this reduced the cross-section of the aorta below the diaphragm by factor of three. Aortic coarctation was induced in the 67 animals of the 4th group and they were also given aminopterin. On the 2nd, 4th, 7th and 21st days after the beginning of the experiment 5-12 rats from each group were killed with ether vapor.

Immediately before injection the aminopterin was dissolved in distilled water so that 1 ml of solution contained 10 μg of the drug; it was injected intraperitoneally in a dose of 60 μg per kg of body weight on the day of the operation, on the following day, and then every other day until the 7th day.

The rate of protein synthesis was evaluated from the incorporation of radioactive methionine (methionine containing S^{35}), which was injected intraperitoneally in a dose of 0.1 μCi per g of body weight 2 h before the animal was killed. After the subject was sacrificed the heart was excised, washed with water, and dried with filter paper. The ventricles were weighed on torsion balances, homogenized, and twice treated with trichloracetic acid, alcohol and ether, and ether. The activity of the radioactive methionine incorporated into 20 mg of the isolated and dried protein was determined over a 10 min period. The relative weight of the ventricles was calculated (by determining the ratio of the corrected weight of the ventricles to the animal's weight before the operation, by the method developed by Alexander et al. [5]).

TABLE 1. Influence of Aminopterin on Protein Synthesis at Various Intervals After The Onset of Compensatory Cardiac Hyperfunctioning

Time after beginning of expt. (in days)	Radioactive methionine incorporation (in pulses/min per 20 mg of protein; M ± m)				Probability of difference			
	Group of animals				P_{3-4}	P_{4-1}	P_{3-1}	P_{2-1}
	1	2	3	4				
2	343±6	246±16	530±10	410±5	<0,01	<0,01	<0,01	>0,1
4	403±8	369±19	580±8	433±7	<0,01	<0,02	<0,01	>0,1
7	291±8	304±17	388±8	347±11	<0,02	<0,01	<0,01	>0,1
21	176±5	172±6	207±13	206±7	>0,1	<0,01	<0,05	>0,1

TABLE 2. Influence of Aminopterin on The Development of Myocardial Hypertrophy at Various Intervals after The Onset of Compensatory Cardiac Hyperfunctioning

Time after beginning of expt. (in days)	Relative wt. of ventricles ($M \pm m.10^5$)				Probability of difference			
	Group of animals				P_{3-4}	P_{4-1}	P_{3-1}	P_{2-1}
	1	2	3	4				
2	286±8	300±10	353±13	320±7	<0,05	<0,01	<0,01	<0,05
4	284±7	299±7	378±16	317±12	<0,02	<0,05	<0,01	>0,1
7	295±6	306±12	379±8	348±7	<0,02	<0,01	<0,01	>0,1
21	299±7	316±8	435±30	417±12	>0,1	<0,01	<0,01	>0,1

EXPERIMENTAL RESULTS

Aminopterin inhibits protein synthesis and increases the mass of the heart in compensatory cardiac hyperfunctioning (Tables 1 and 2).

In the animals of the 3rd group radioactive methionine incorporation was 54% above normal 2 days after coarctation, 44% above normal on the 4th day, 32% above normal on the 7th day, and 17% above normal on the 21st day.

Radioactive methionine incorporation was retarded in the animals of the 4th group in comparison with those of the 3rd group, especially at short intervals—2-4 days (see Table 1).

The changes in relative ventricle weight in the animals of the 3rd and 4th groups were similar to those in radioactive methionine incorporation. Thus, in the animals of the 3rd group relative ventricle weight was 31% above normal on the 2nd day, 32% above normal on the 4th day, 28% above normal on the 7th day, and 45% above normal on the 21st day. In the animals of the 4th group relative ventricle weight was elevated by only 15% on the 2nd day, 12% on the 4th day, 18% on the 7th day, and 39% on the 21st day (see Table 2).

It must be noted that the difference between the 3rd and 4th groups with respect to both radioactive methionine incorporation and relative ventricle weight became statistically reliable by the 21st day.

The changes in radioactive methionine incorporation and relative ventricle weight were slight in the animals of the 2nd group, except on the 2nd day after the experiment began, when the relative ventricle weight was found to be elevated by 12%.

The inhibition of cardiac hypertrophy induced in the rats with aortic coarctation by the DNA-synthesis inhibitor caused 83% of the animals to die of cardiac insufficiency, exhibiting symptoms of hydrothorax and ascites. Death from cardiac insufficiency occurred in only 36% of the animals with aortic coarctation which did not receive aminopterin.

The fact that administration of aminopterin did not cause lethal cardiac insufficiency in the animals not subjected to aortic coarctation is important.

In discussing the results obtained it must be kept in mind that the drug which we employed, blocking dihydrofolic reductase, disrupts the conversion of folic acid to tetrahydrofolic acid. This causes a disturbance of thymidyl triphosphate formation and halts DNA synthesis [9].

As our later experiments showed, inhibition of RNA synthesis is observed simultaneously with the inhibition of protein synthesis. There are two different hypotheses to explain this phenomenon. First, it is possible that the cessation of DNA synthesis under the influence of aminopterin leads to inhibition of RNA and protein synthesis, since newly formed DNA is necessary for intensive activation of this process. Secondly, we cannot exclude the possibility that the primary effect of aminopterin is not limited to inhibition of DNA synthesis, as is commonly supposed, but extends directly to RNA and protein synthesis. Experimental verification of these hypotheses is one of the goals of our further work.

Of substantial interest is the attenuation of the action of aminopterin on radioactive methionine incorporation into cardiac protein and on relative ventricle weight which we observed from the 7th day of the experiment onward, a phenomenon which conflicts with the fact that Charache et al. [7] detected a folic acid antagonist in mice and humans 100 days after its administration. Considering that researchers [8, 10, 11] have detected an increase in dihydrofolic reductase content during acclimatization of mouse leukemia cells and sarcoma 180 cells to aminopterin, the attenuation of the action of aminopterin observed in our experiments may possibly be due to an increase in dihydrofolic reductase content.

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