circumstance is fundamentally important, for it helps maintain the functional activity of energy-dependent mechanisms of ionic homeostasis in cardiomyocytes and determines the gradation of glycolysis intermediates by their antiarrhythmic activity as a function of the energetic value of the substrate.

Of course, this hypothesis does not exhaust all the aspects in the mechanism of the antiarrhythmic effect of glycolysis intermediates during early postocclusion arrhythmias. For instance, the antiacidotic effect of FDP and PEP [4] may be of importance, because metabolic acidosis is one of the key components in the pathogenesis of arrhythmias occurring in acute myocardial ischemia [3,7].

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Effect of Polycation Based on Alkaloid Lupinin-Antihepolin and Its Complex with Heparin on DNA Synthesis in Rat Hepatocytes

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The effect of the antiheparinate antihepolin on the intensity of replication processes in rat hepatocytes was studied. A single intravenous infusion of polycation caused an approximately 6-fold increase in the intensity of DNA synthesis on day 2 postinjection followed by a drop to thebaseline level on days 5-6. If antihepolin was injected in parallel with heparin, DNA synthesis was intensified after just 24 hours.

Key Words: *polycations; heparin; antihepolin; polycomplex; hepatocytes; DNA synthesis*

Among synthetic polymers characterized by antiheparin activity linear ionenes are known, such as polybrene [4,3], quaternary ammonium salt of oligomer 25 conidine (QAS-O-25 conidine) [7], and other substances of polycationic structure [2]. Synthetic polycations deserving special attention are those in which natural substances are used as quaternized nitrogen carriers in their construction. For example, a systematic series of polymers that are quaternary salts of polymethacryloil lupinin (poly-MACL) [5] was synthesized on the basis of an alkaloid of *Anabasis aphylla,* lupinin, whose tertiary nitrogen atoms may be easily transformed into quaternary atoms [6]. A characteristic feature of

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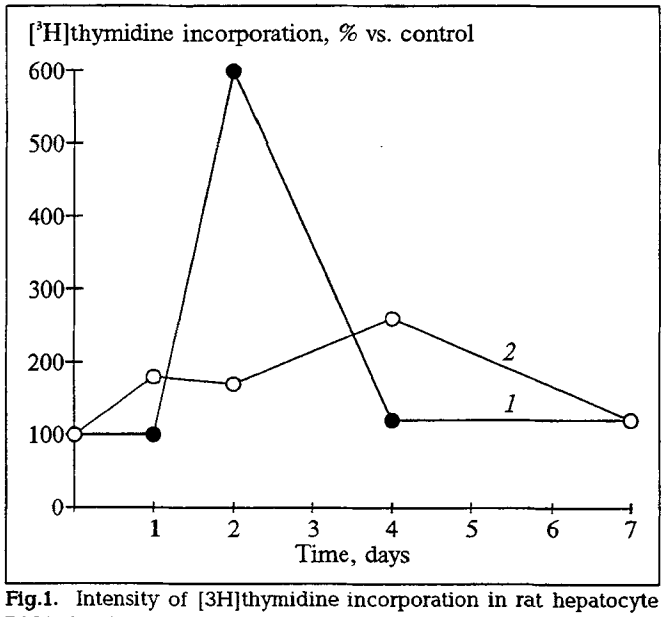

Fig.1. Intensity of [3H]thymidine incorporation in rat hepatocyte DNA for intravenous injection of AH (1) or heparin and AH (2) (in % *vs.* control)

their structure is the position of carriers of charged groups outside the main chain of the polymer, that is, in the lateral macrochain groupings. Pharmacological studies of polycations with such a structure with average molecular weight (m.w.) from monomer to polymer and number of monomeric component replicas n=100 and m.w. 47,000 revealed [9] that n=50 (m.w. 20,000) was the optimal value of polymerization degree in the studied series. This compound, under the draft name antihepolin (AH), is characterized by a favorable combination of high antiheparin activity and low toxicity. Pharmacodynamic studies of this compound showed that, like other studied polycations, it is retained mainly in the liver. But in contrast to synthetic polycations, AH is quite rapidly destroyed in the body and is virtually completely eliminated within 10 days after infusion into the bloodstream [12]. Linear synthetic polycations (e.g., QAS O-25 conidine) have been shown to be retained in the liver for a long time, the more so after interaction with heparin [10].

TABLE 1. Intensity of [³H]Thymidine Incorporation (imp per mg DNA) in DNA of Rat Liver Homogenate in Group 1 and Group 2

Time, days	Group of animals	
	1	2
1	3.46	3.50
2	3.01	17.93*
4	9.01	9.92*
7	4.26	4.90*

Note. Here and in Table 2: n=3; asterisk shows values reliable at p<0.05.

They are localized in the hepatocyte genome, in which they competitively replace natural positively charged proteins from their natural complex with DNA. This changes the secondary structure of the DNA molecule and the spatial arrangement of chromatin and influences the intensity of replication, transcription, and translation processes in the animal organism [11]. It is possible that a polycation synthesized on the basis of alkaloid lupinin may activate matrix biosynthesis similarly as QAS O-25, but the duration and specific features of this effect of AH and its complex with heparin are unknown.

The purpose of this study was to investigate the intensity of replication process in rat hepatocytes under conditions of AH neutralization with heparin and spontaneous infusion of polycation into the bloodstream.

MATERIALS AND METHODS

Male rats weighing 140 to 160 g were used in the experiments. Liver tissue was taken on days 1, 2, 4, and 7 postinjection. The animals were divided into 3 groups: group 1 were controls, group 2 were intravenously injected AH in a dose of 5.4 mg/kg, and in group 3 heparin was intravenously injected in a dose of 5 mg/kg, followed 10 min later by a neutralizing amount of AH [9]. For assessment of the intensity of replication all the animals were intraperitoneally injected [³H]thymidine (100 μCi per 100 g) 1.5 h before decapitation. After decapitation the liver was removed and weighed. All subsequent operations were carried out at 0-4°C. Liver tissue was minced and homogenized in 5 volumes of medium with 0.03 mmol Tris-HCl, pH 8.0, 0.25 mmol KCl, and 0.05 mmol $Mg(CH_3COO)_2$ in a homogenizer with Teflon pestle. The homogenate was filtered to discard the larger particles and its volume measured. DNA was isolated after a modified Schmidt and Tanhauser's method [8]. The intensity of DNA synthesis was assessed from [³H]thymidine incorporation in the acid-insoluble fraction converted to 1 mg of DNA. Results were statistically processed using Wilcoxon-Mann-Whitney's test (the results are presented as the medians).

RESULTS

Table 1 shows that the intensity of DNA synthesis in the animals injected AH alone was the same as in controls 1 day after injection.

However, as soon as on day 2 activation of [3H]thymidine incorporation in DNA was observed in this group of animals. It was approximately 6 times higher than the rate of the replication pro-

cess in the controls. The mechanism of increase of replicative activity in this case may be explained by the entry of the agent into the hepatocyte nucleus (by analogy with QAS O-25 conidine) and subsequent changes in the degree of chromatin condensation under the effect of positively charged macromolecules. The drop in the intensity of [3H]thymidine incorporation to baseline levels observed during the next 5 days was caused by a gradual disintegration and elimination of AH from the body.

When AH was injected after heparin, the rate of replication was increased 24 h after injection of both polyelectrolytes (Table 2). The intensity of DNA synthesis during this period was approximately twice as high as than in animals injected AH alone, and this level remained unchanged for 3 days. This may be due to the fact that heparin, once having formed an interpolyelectrolyte complex with AH, is incapable of blocking all the charges of polycation of this structure. As a result, thepolycomplex possesses several free positive charges which cannot provide for reliable interaction with cytoplasm components impeding migration of thepolycomplex in the cytosol towards the nucleus, but these charges are sufficient for rapid passage of the polycomplex through thenuclear membrane. Hence, during the first day the number of carriers of cationic charge entering the cell genome in these animals is higher than after injection of AH alone. The appearance of positively charged structures in the nucleus immediately activates replication.

The difference in intensity of replication in animals of the 2nd and 3rd groups during the first 3 days (Fig. 1) may be attributed to specific features of biotransformation of the interpolyelectrolyte complex heparin-AH, in which destruction of the heparin component is more rapid. It is caused by liver heparinase [10]. Not yet destroyed cationic components which are released and the still intact polycomplexes continue stimulation of matrix activity. Only after complete destruction of the heparin integrated in the complexes does it become possible for AH to intensively interact with a potent cellular polyanion - DNA molecules. Synthesis of nucleic acids must be activated during this period, and this does indeed take place on day 3 in hepatocytes of group 3 animals. The intensity of DNA synthesis increases, reaching the maximum (260% vs. the control) on day 4. However, the maximal activity of DNA synthesis on day 4 and later was lower in group 3 than on days 2-3 in group 2. This is most probably due to partial destruction of AH in the course of disintegration of the whole heparin-AH polycomplex. In group 2 a surge of replication observed on day 2 is followed by reduction of DNA synthesis

TABLE 2. Intensity of [^{3}H]Thymidine Incorporation (imp per mg DNA) in DNA of Rat Liver Homogenate in Group 1 and Group 3

Time, days	Group of animals	
	1	3
1	2.10	9.65*
2	2.39	3.98*
4	0.79	2.53*
7	2.72	2.73

activity to baseline levels. Hence, AH rather quickly loses the capacity to interact with DNA, which may be a result of the loss of the property of multiple positive charge in a macromolecule. When the effect of quaternary polyammonium salts ceases, the normal level of DNA synthesis and cell division recovers, this indicating the intactness of theintracellular regulatory system controlling the processes of synthesis. It is confirmed by the data on the effects of natural positively charged compounds, polyamines, on the intensity of matrix processes [1].

Hence, AH influences the processes of replication by enhancing and synchronizing DNA synthesis. The increased intensity of DNA synthesis observed during the first day after the formation of a heparin-AH polycomplex in the bloodstream is caused by its cationic component. Withdrawal of the studied polyelectrolytes from the hepatocyte genome is associated with recovery of the normal level of DNA synthesis activity.

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