HEPARIN CLEARANCE DURING EXCITATION OF THE ANTICLOTTING SYSTEM IN ANIMALS

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During excitation of the anticlotting system (by means of thrombin) in animals the clearance of [^{35}S]heparin from the blood is delayed. The half-life of [^{35}S]heparin increases under these circumstances from 1.6 h in control animals to 2.35 h in animals receiving intravenous injections of threshold doses of thrombin. The slowing of [^{35}S]heparin clearance from the blood during excitation of the anticlotting system is accompanied by an increase in its absorption in the liver and in the auricles of the heart.

KEY WORDS: heparin; thrombin; anticlotting system.

Heparin, the most important humoral agent of the anticlotting system (ACS), plays a key role in the cycle of processes aimed at preventing and removing the threat of thrombosis. Excitation of the ACS is accompanied by the release of endogenous heparin from tissue sources, to form complexes with certain plasma proteins and biogenic amines. These complexes have well-marked ability to dissolve fibrin clots unstabilized by factor $XIII_a$, as a result of which the circulating blood is kept in a liquid state [1, 10, 11]. Heparin, by forming a complex with thrombin, leads to the more rapid clearance of thrombin from the blood stream and its deposition in the form of a thrombin–heparin complex chiefly in the liver tissue [2, 3, 5, 6, 12]. Heparin complexes formed in the auricles of the heart have been shown to play an important role in maintenance of the normal blood flow in the heart. It has also been shown that the fraction of so-called fibrin degradation products, eliminated from the blood after excitation of the ACS by threshold doses of thrombin, consists mainly of a complex of heparin with fibrinogen [4].

The object of this investigation was to study the initial stages of [^{35}S]heparin clearance in animals during excitation of the ACS by thrombin.

EXPERIMENTAL METHOD

Experiments were carried out on 84 male albino rats weighing 180-200 g. [^{35}S]Heparin (from the Radiochemical Centre, Amersham), with a radioactivity of 1 μCi and biological activity of 2-3 i.u./200 g weight, was injected intravenously into the animals. The ACS was excited by intravenous injection of 50 units thrombin (from the Kaunas Bacterial Preparations Factory); control animals received an injection of physiological saline. The test substances were injected in a volume of 1 ml physiological saline. Intravenous injections were given into and blood was taken from the jugular vein. The blood was mixed with Na citrate in a volume of not more than 0.2 ml. The blood samples and weighed samples of tissues for testing were treated by Herberg's method [9]. Radioactivity was measured with a Mark II (Nuclear Chicago, USA) liquid scintillation counter. The half-life of [^{35}S]heparin in the animals was determined graphically by Sterling's method [14]. The results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

Investigation of the initial stage of clearance of [^{35}S]heparin in the control animals receiving injections of physiological saline instead of thrombin showed that the half-life (T/2) of the labeled heparin was 1.6 h. The quantity of [^{35}S]heparin detected in the blood stream 4 h after intravenous injection was shown to be 7.5% of the quantity detectable 1 min after its injection (Table 1). The largest quantity of accumulated heparin was observed in the liver tissue. For instance, after 2 h the specific radioactivity of the liver tissue was 118.8% of

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TABLE 1. Specific Radioactivity of Blood Due to [^{35}S]Heparin as a Ratio of Specific Radioactivity of Blood 1 min After Injection of [^{35}S]Heparin (in %)

Experimental conditions	Statistical indices	Time after injection of [^{35}S] heparin, min								
		1	5	10	20	30	60	120	180	240
Control	M	100	60,3	36,8	24,8	17,5	10,9	5,5	5,9	7,5
	$\pm m$		5,6	3,6	2,9	2,0	1,7	1,4	1,5	0,8
	n		6	8	7	8	11	9	6	4
Injection of thrombin solution (experiment)	M	100	89,0	65,2	59,3	32,0	28,0	14,4	15,4	13,3
	$\pm m$		7,2	5,1	4,0	2,1	3,3	1,8	1,5	1,6
	n		8	6	8	6	8	5	4	5

TABLE 2. Specific Radioactivity of Organs Due to [^{35}S]Heparin as a Ratio of Specific Radioactivity of Blood 1 min After Injection of [^{35}S]Heparin (in %, M ± m)

Experimental conditions	Organs	Time after injection of [^{35}S] heparin, min						
		10	20	30	60	120	180	240
Control	Liver	41,3±2,1	61,9±5,1	131,4±11,8	98,2±6,2	118,8±6,3	73,9±4,9	81,2±9,2
	Lungs	22,2±0,9	18,0±1,2	14,4±1,7	11,3±0,7	9,3±1,4	7,7±1,1	5,6±0,8
	Spleen	21,6±1,7	29,1±2,7	37,5±2,2	37,8±2,4	34,6±4,2	36,6±5,1	32,1±5,0
	Heart	16,5±1,4	14,9±0,8	11,2±0,7	8,1±2,0	7,1±2,0	5,7±0,4	7,0±0,7
	Auricles	13,3±1,7	12,6±0,9	10,4±1,1	9,0±1,7	8,5±2,1	7,0±0,6	6,1±0,9
		(n=6)	(n=6)	(n=6)	(n=7)	(n=5)	(n=6)	(n=5)
Injection of thrombin solution (experiment)	Liver	66,6±3,4	85,3±6,2	103,5±7,3	105,7±6,9	158,5±12,4	158,7±11,4	166,2±13,7
	Lungs	28,7±4,1	21,6±2,0	34,4±3,7	26,2±2,8	19,3±3,0	10,7±1,9	16,1±3,1
	Spleen	26,0±3,9	30,5±4,0	48,4±5,2	33,2±2,7	38,0±4,1	52,7±7,4	27,1±2,1
	Heart	15,7±1,2	16,8±1,6	14,2±2,2	12,8±0,8	12,2±1,5	11,5±1,1	12,3±2,7
	Auricles	25,3±1,4	20,7±1,7	26,8±1,9	27,0±2,1	20,1±1,8	13,5±1,0	12,5±1,6
		(n=6)	(n=6)	(n=6)	(n=6)	(n=6)	(n=6)	(n=6)

the specific radioactivity of the blood after the first minute of the experiment. After 120 min the accumulation of [^{35}S]heparin in the liver was reduced to 73.9-80.2% (Table 2). Products of metabolic degradation of heparin, entering the blood stream, were evidently responsible for the observed stabilization of the blood [^{35}S]heparin level between 120 and 240 min of the experiment. Some degree of absorption of labeled heparin was discovered in the tissues of the spleen: up to 37.8% after 60 min (Table 2).

Investigation of the [^{35}S]heparin clearance during excitation of ACS showed that under these conditions there was an increase in T/2 to 2.35 h ($P < 0.001$). The total blood level of labeled heparin throughout this period of the experiment was 1.5-2.6 times higher than its level in the blood of the control animals (Table 1). However, the over-all slowing of [^{35}S]heparin clearance from the blood during excitation of the ACS by thrombin was accompanied by a higher rate of its accumulation in the liver (up to 166.2% after 240 min). Some increase in the absorption of labeled heparin in the lung tissues also was found. During excitation of ACS accumulation of heparin also was found in the auricles of the heart. For instance, after 30-60 min the heparin concentration determined in the auricles was twice as high as in the heart muscle (Table 2). This fact, already established by the writers previously, is evidence of the important role of heparin and its complexes in the maintenance of the normal blood flow in the heart itself when thrombosis is threatened.

The data for the half-life of [^{35}S]heparin agree with the results obtained by other workers who determined this index in mammals of different species and found it to be between 17.5 min and 1.4 h [7, 8, 13]. The increase in T/2 in animals during excitation of their ACS, discovered in the present experiments, can be explained by intensification of the formation of complexes of heparin with proteins and amines, thereby ensuring a longer circulation of [^{35}S]heparin, bound in complexes, in the blood stream. At the same time, however, what can be regarded as a paradoxical fact, namely an increase in its absorption in the liver, was observed. This can be explained on the basis of previous investigations [2, 3, 5, 6, 12] which showed that during excitation of the ACS thrombin from the blood stream accumulates rapidly in the liver tissue, mainly in the form of a thrombin–heparin complex.

During excitation of the ACS heparin, in the form of its complexes [1, 11], is thus retained in the circulation and accumulates in the tissues of the lungs and heart, where it causes lysis of fibrin clots which have succeeded in forming and thereby maintains the liquid state of the blood in the body. On the other hand, by binding thrombin [12], it promotes its rapid removal from the blood and accumulation in the liver tissue, thereby removing the direct threat of thrombus formation.

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