in this case were: R_0 = 0.15 mm Hg/ml/min; C = 6.5 μl/mm Hg; R_1 = 0.85 mm Hg/ml/min; P_{zf} = 35 mm Hg; K_2 = 0.3, and K_3 = 0.35.

Analysis of the data showed that, on substitution of parameters characteristic of the coronary circulation, better qualitative agreement was obtained between the theoretical and experimental coronary blood flow curves. With fewer parameters, in models suggested previously [2, 5-8], this agreement could not be obtained.

The model suggested in [2, 6] was intended to describe the diastolic flow characteristics, whereas the behavior of the coronary blood flow during systole and some of the effects of compliance [4, 7, 9] were not fully described. Another model [7] was constructed on the basis of intramyocardial capacity. This model explains the increase in coronary blood flow during systole and certain other effects, but in principle it cannot explain the phenomenon of the linear flow characteristic and the zero flow pressure (P_{zf}).

Our suggested model is free from these disadvantages. The results obtained with it showed that it adequately describes the shape of the coronary blood flow curve. Later it is planned to study the possibility of solving the opposite problem, i.e., of finding the parameters R_0, S, R_1, P_{zf}, K_2, and K_3 of the coronary circulation from the curves of pressure in the aorta and left ventricle and of the coronary blood flow.

As a first approximation this model may enable multicomponent analysis of coronary vascular reactions. Nevertheless, it probably needs further improvements and, in particular, the possibility of allowing for nonlinearity of behavior of individual parameters.

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ANTICOAGULANT AND ANTITHROMBOTIC EFFECTS OF LOW-MOLECULAR-WEIGHT HEPARIN

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KEY WORDS: heparin; antithrombin III, thrombosis.

There is evidence in the literature that, besides their positive effects, commercial forms of heparin also may give rise to various side effects, such as hemorrhage, thrombocytopenia, and osteoporosis, and they may also stimulate aggregation of platelets and erythrocytes [12]. The search thus continues for heparin preparations free from the above-mentioned disadvantages. The composition of heparin, which is a mixture of glycosaminoglycans with different degrees of polymerization (mol. wt. 5-40 kD) is such that it is possible to obtain preparations with preassigned properties. The use of a low-molecular-weight heparin (LMH), with mol. wt. of under 10 kD, has been reported [5]. Compared with the commercial preparation, LMH has weaker anticoagulant activity but it is a more effective antithrombotic agent in vivo and gives rise to significantly fewer side effects [13].

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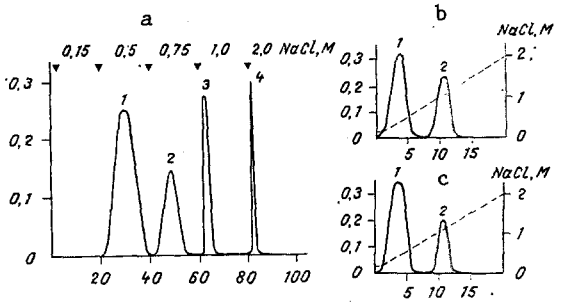

Fig. 1. Chromatography of heparin on DEAE-Sephadex (a) and on antithrombin III-sepharose (b, c). Abscissa, No. of sample; ordinate, absorbance of samples (A_{470}) on determination of heparin by a turbidimetric method. a) On column with DEAE-Sephadex A-50 (2.5 × 60 cm), equilibrated with 0.1 M Tris-HCl buffer (pH 7.4), containing 0.15 M NaCl, 100 mg of heparin in the same buffer was added. Heparin was eluted by a stepwise ionic strength gradient created by increasing NaCl concentrations in the same buffer. Rate of elution 50 ml/h, 5-ml samples collected. 1, 2, 3, 4) Components 1, 2, 3, and 4 respectively; b, c) to column with antithrombin III-sepharose (0.5 × 7 cm), equilibrated with 0.1 M Tris-HCl buffer (pH 7.4), containing 0.15 M NaCl, 500 μg of heparin (b) or LMH (c) in the same buffer was added. Heparin was eluted by a linear NaCl concentration gradient (0.15-2 M) in the same buffer. Rate of elution 30 ml/h, 1-ml samples collected. Broken line indicates NaCl concentration (in M). 1, 2) Components 1 and 2 respectively.

The aim of the present investigation was to obtain LMH and to study its anticoagulant and antithrombotic properties in animals with a normal and depressed antithrombin III level.

EXPERIMENTAL METHOD

Heparin (156 USP U/mg; from "Sigma," USA) was fractionated by chromatography on DEAE-Sephadex A-50 ("Pharmacia," Sweden) by the method in [11]. Heparin was eluted by a stepwise ionic strength gradient created by increasing NaCl concentrations (0.15, 0.5, 0.75, 1, and 2 M) in 0.1 M Tris-HCl buffer (pH 7.4). The heparin concentration in the eluates was determined turbidimetrically [7]. Heparin was precipitated with ethanol and lyophilized. The molecular weight of the heparin and its fractions was determined by gel-filtration on a column with Sephadex Ce-100 ("Pharmacia"), using LMH with mol. wt. of 5.5 kD ("Calbiochem," West Germany) and dextrans with mol. wt. of 15-20 and 40 kD ("Loba-Chemie," Austria) as the standards. Dextrans were determined by the method in [4]. The ability of heparin and its fractions to interact with antithrombin III was studied by affinity chromatography for antithrombin III—sepharose, synthesized by the method in [2]. Antithrombin III was isolated from bovine blood plasma by biospecific chromatography on heparin—sepharose [8, 9]. Antithrombin III, homogeneous on electrophoresis in PAG—SDS, with a molecular weight of 68 kD, and specific antithrombin activity of 6 U/mg, was immobilized on BrCN-sepharose 4B ("Pharmacia") by a method recommended by that firm. The anticoagulant activity of heparin and its fractions was determined by determination of the activated partial thromboplastin and thrombin times [1]. The anti-IIa activity of the heparin preparations was studied by determining inhibition of the amidase activity of α-thrombin by antithrombin III relative to phenylalanyl-pipecolyl-arginine paranitroanilide ("Serva," West Germany) in the presence of different heparin concentrations [10]. Anti-Xa activity of heparin was studied by the method in [14]. Unfractionated heparin (156 USP U/mg; "Sigma"), for determination of anticoagulant and anti-IIa activity of heparin, and LMH (mol. wt. 5.5 kD; 90 anti-Xa U/mg; "Calbiochem"), for determination of anti-Xa activity, were used as the standards. Experiments in vivo were carried out on noninbred albino rats weighing 180-220 g, in some of which an experimental nephrotic syndrome (Heymann's nephritis) was induced as described previously [1]. In the animals with a nephrotic syndrome the urinary protein and blood urea concentrations were determined by unified methods, the concentration of soluble fibrin—monomer complexes as in [1], antithrombin III activity by blockade of the amidase activity of thrombin relative to CHG-clycyl-arginine paranitroanilide by the "M+D Antithrombin III" kit ("Merz + Dade AG," Switzerland). The biological half-life of heparin and LMH was determined by the method in [6]. The antithrombotic action of heparin and LMH was studied on a model of thrombosis in an artereovenous shunt [3], with an original recording

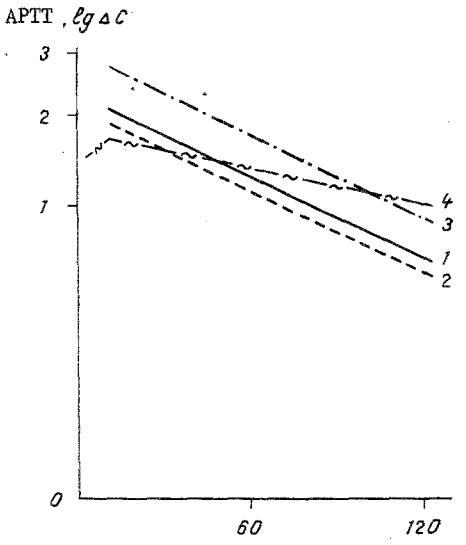

Fig. 2. Semilogarithmic plot of changes in activated partial thromboplastin time (APTT) after intravenous injection of unfractionated heparin and LMH. Abscissa, time after injection (in min); ordinate, log Δ APTT, calculated by the formula log Δ APTT = $APTT_t - APTT_0$, where $APTT_0$ is the value of the parameter (in sec) before injection of the preparations, and $APTT_t$ its value (in sec) at different times after injection of the preparations. 1, 2, 3) Unfractionated heparin in doses of 10, 20, and 50 USP U/200 g body weight respectively; 4) LMH in a dose of 20 USP U/200 g body weight. Parameters were calculated after injection of different doses of preparations into groups of animals consisting of three or four rats.

method. By contrast with the basic method, the time of thrombus formation was determined by the fall of blood pressure in the shunt, measured by a "Statham" transducer (USA), with parallel recording of the blood pressure on an N-338 III automatic writer (USSR) and display on a V7-35 digital indicator (USSR). The rats were anesthetized by intraperitoneal injection of hexobarbital in a dose of 100 mg/kg. The experimental results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Fractionation of heparin by ion-exchange chromatography on DEAE-Sephadex showed that at physiological ionic strength (0.15 M NaCl) the whole of the glycosaminoglycan was bound with cationic groups of the carrier. If the ionic strength was increased by creation of a stepwise NaCl concentration gradient, the heparin separated into four components (Fig. 1a): component 1 was desorbed from the matrix by NaCl in a concentration of 0.5 M and contained 56% of the heparin eluted from the column; component 2, containing 20% of heparin, was eluted by NaCl in a concentration of 0.75 M; components 3 and 4 (15 and 9% of heparin respectively) were eluted by NaCl in concentrations of 1 and 2 M respectively. According to gel filtration on Sephadex G-100 the average molecular weight of the heparin fraction eluted in component 1 was 8 kD (7-14 kD). This fraction will subsequently be called LMH. The average molecular weight of the heparin fraction eluted in component 2 was 28 kD (17-48 kD), and that of the unfractionated heparin was 18 kD (8-47 kD). The composition of the minor components 3 and 4 was not analyzed. On chromatography of commercial heparin on DEAE-Sephadex an LMH fraction containing 56% of the heparin eluted from the sorbent was thus obtained.

Since the heparin preparations were extremely heterogeneous as regards their affinity for antithrombin III, the ability of commercial heparin and LMH to interact with immobilized antithrombin III was investigated. On fractionation of the heparin preparations on antithrombin III-sepharose, they separated into two components with different affinity for the immobilized ligand: component 1 with low affinity for antithrombin III, containing 66% of the heparin (Fig. 1b) and 75% of the LMH (Fig. 1c), was eluted by NaCl in a concentration of 0.15-0.5 M; component 2, with high affinity for antithrombin III, was desorbed from the matrix by NaCl in a concentration of 0.9-1 M and contained 34% of the heparin (Fig. 1b) and 25% of the LMH (Fig. 1c) respectively. The results of chromatography indicate a fall in the content of the fraction with high affinity for antithrombin III in LMH.

TABLE 1. Changes in Thrombus Formation Time in Arteriovenous Shunt in Intact Animals and Rats with Nephrotic Syndrome after Injection of Unfractionated Heparin and of LMH in a Dose of 20 USP U/200 g Body Weight

Preparation	Time after injection, min	Thrombus formation time, min	
		intact rats	rats with nephrotic syndrome
0.9% NaCl solution	20	2,4±0,2 (11)	1,5±0,4 (3)
p		—	<0,01
Unfractionated heparin	20	4,9±0,1 (9)	30,7±0,5 (3)
p		<0,001	<0,001
LMH	30	9,9±0,1 (5)	45,0±0,2 (3)
p		<0,001	<0,001
p_1		<0,001	<0,001

Legend. In group of animals with nephrotic syndrome and receiving 0.9% NaCl solution, significance of differences was calculated relative to intact animals receiving 0.9% NaCl solution. p) Significance of differences between groups of animals receiving 0.9% NaCl solution and heparin preparations; p_1) significance of differences between groups of animals receiving unfractionated heparin and LMH.

Investigation of the anticoagulant activity of LMH by the activated partial thromboplastin time (APTT) method showed that it was 95% lower ($p < 0.01$) than that of the unfractionated preparation. The anticoagulant activity of LMH was 8.2 ± 1 USP U/mg. According to the results of determination of thrombin time, the anticoagulant activity of LMH was 39 ± 3 USP U/mg. The anti-IIa activity of LMH was 72 ± 5 USP U/mg compared with 156 ± 5 USP U/mg for commercial heparin. The anti-Xa activity of LMH was 217 ± 4 U/mg and that of the unfractionated preparation was 186 ± 5 U/mg. Thus LMH had lower affinity for antithrombin III than the commercial preparation and, as a result of this, it had lower anticoagulant and anti-IIa activity. The anti-Xa activity of LMH was 17% higher than that of unfractionted heparin ($p < 0.01$).

The study of the kinetics of elimination of unfractionated heparin and LMH showed that the half-life of commercial heparin was 78 ± 10 min whereas that of LMH was 190 ± 20 min (Fig. 2). At all times, more marked lengthening of APTT was observed.

The antithrombotic action of heparin and LMH was compared on a model of thrombus formation in an extracorporeal arteriovenous shunt. In the control series of experiments on intact rats receiving 0.5 ml of 0.9% NaCl solution, 2.4 ± 0.2 min after the beginning of the blood flow there was a sharp fall of blood pressure in the shunt by 50 ± 10 mm Hg, due to its thrombotic occlusion (Table 1). Thrombus formation was prevented by intravenous injection of unfractionated heparin and LMH in a dose of 20 USP U/200 g body weight. The blood flow in the shunt was restored 20 min after injection of the commercial preparation. Under the influence of unfractionated heparin the thrombus formation time was increased by 75% ($p < 0.001$). Considering the longer half-life of LMH in the blood stream, the circulation in the shunt was resumed 30 min after injection of LMH. Under these conditions the thrombus formation time was lengthened by LMH by 312% ($p < 0.001$), i.e., with the same dose injected, LMH increased the thrombus formation time more than twofold compared with the unfractionated preparation.

By the use of models of experimental thrombosis and the nephrotic syndrome, the antithrombotic action of heparin preparations could be evaluated under conditions simulating those of hemodialysis, accompanied by activation of the contact stage of blood clotting and of the platelets. A nephrotic syndrome characterized by proteinuria (1.2 ± 0.2 mg/ml; $p < 0.001$)

and by an increase in the blood urea concentration to 8.4 ± 0.3 mM ($p < 0.001$) was observed 3 months after injection of the immunogen into the rats. The nephrotic syndrome was accompanied by a prethrombotic state, demonstrated by a 4.5-fold increase in the concentration of soluble fibrin-monomer complexes ($p < 0.001$) and an acquired antithrombin III deficiency. The plasma antithrombin III activity was reduced by 30% ($p < 0.01$). Compared with intact animals, the thrombus formation time in rats with the nephrotic syndrome was reduced by 30% ($p < 0.001$; Table 1). Under these conditions unfractionated heparin prevented thrombus formation for 30 ± 0.5 min ($p < 0.001$). Compared with the commercial preparation, LMH increased the thrombus formation time in the shunt by 45% ($p < 0.001$).

These results are evidence that LMH, with its predominantly inhibitory action on factor Xa, is a more effective antithrombotic agent both when the antithrombin III level in the body is normal and when it is lowered. The high antithrombotic activity of LMH is combined with a long period of its retention in the blood stream, so that the dose of the preparation required for the prevention of thrombosis can be reduced.

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NEUROPHYSIOLOGICAL STUDY OF EFFERENT–AFFERENT INTERACTION ON PARIETAL ASSOCIATION CORTICAL NEURONS IN CATS

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KEY WORDS: parietal cortical neurons; pyramidal tract; convergence of excitations; learning.

In the modern view the parietal association cortex not only is involved in the realization of afferent functions of interanalyzer synthesis, but also participates in programming of the efferent, motor functions of the brain [2-4, 8]. Integrated afferent information is transmitted from the parietal to the motor cortex along channels of direct communication between the neuron complexes of the parietal cortex and the neuron pools of the motor cortex, controlling the corresponding peripheral effectors [7, 9].

The aim of the present investigation was to look for the presence of feedback between the motor and parietal areas of the brain. The morphological substrate for this feedback could be recurrent collaterals of axons of cortical motoneurons. The discovery of feedback between the motor and parietal areas and the elucidation of its functional significance could help to revise our interpretation of the neurophysiological status of the integrative neurons of the parietal association cortex in the programming of motor behavioral acts.

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