basis of the dual dynamics of the change in NLC under the influence of VP. Probably one of the basic mechanisms of the change in amplitude of NLC under the influence of the preparations is opening, blocking, or inactivation of chemosensitive ionic channels [3].

Thus VP has a significant effect on the properties of electrically sensitive ionic channels of the neuron membrane. The important point is that VP, in these same concentrations, effectively activates chemosensitive extrasynaptic neuron membrane receptors. It is possible that under the influence of VP, cooperative relations begin to form between these two systems regulating excitability of the neuron membrane, aimed at securing the most effective possible regulation of cellular excitability.

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EFFECT OF HEPARIN AND THROMBOPLASTIN ON THE HALF-LIFE OF ^{125}I-PROTEIN C IN CIRCULATING RAT BLOOD

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KEY WORDS: protein C; hypocoagulation; hypercoagulation

Protein C is a vitamin K-dependent protein of the blood clotting system which circulates in the blood in the form of the proenzyme — a serine proteinase precursor [12]. Conversion of protein C into its active form (activated protein C) takes place in the body under the influence of thrombin [6]. Activation of protein C is accelerated by several orders of magnitude by the endothelial membrane protein thrombomodulin, which forms an equimolar complex with thrombin, which increases the affinity of thrombin for protein C [3]. Unlike other vitamin K-dependent proteinases of the blood clotting system, namely factors II, VII, IX, and X, which are procoagulants, activated protein C exhibits anticoagulant and profibrinolytic properties. The anticoagulant effect is due to the fact that activated protein C inactivates factors V (Va) and VIII (VIIIa) and, consequently, it inhibits thrombin generation [7]. The profibrinolytic action of activated protein C is connected with the fact that it interacts with the inhibitor of tissue plasminogen activator, with which this activator exists in the form of a complex. This leads to elevation of the

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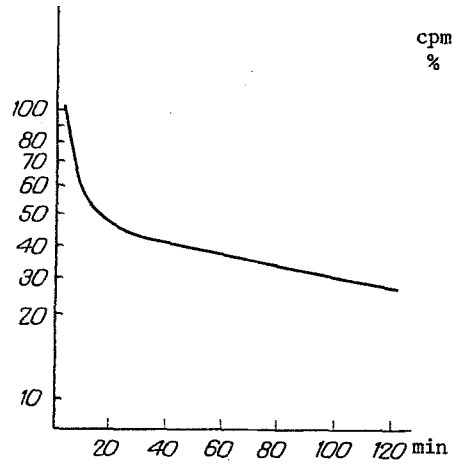

Fig. 1

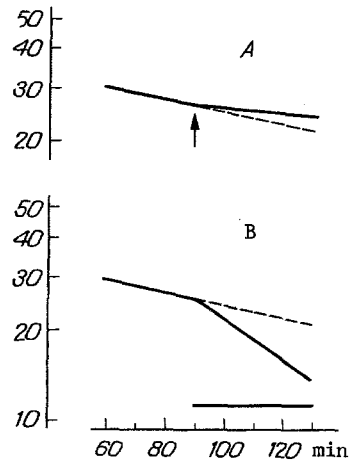

Fig. 2

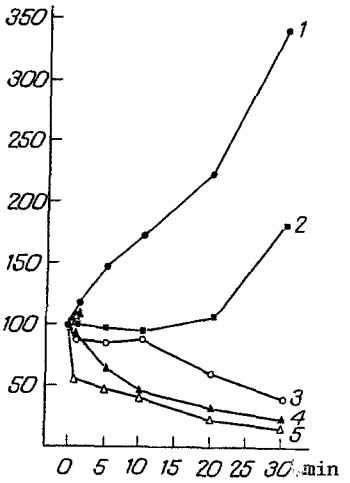

Fig. 3

Fig. 1. Elimination of ^{125}I-protein C from blood stream. Abscissa, time (in min); ordinate, radioactivity of blood samples (in per cent, logarithmic scale).

Fig. 2. Effect of heparin and thromboplastin on elimination of ^{125}I-protein C from blood stream. Abscissa, time (in min); ordinate, radioactivity of blood samples (in per cent, logarithmic scale). I) effect of heparin (injection of heparin indicated by arrow); II) effect of thromboplastin (indicated by horizontal line).

Fig. 3. Changes in Parameters of hemostasis during long-term administration of thromboplastin to rat. Abscissa, time (in min); ordinate, changes in parameters of hemostasis (in per cent of initial value). 1) APTT; 2) thrombin time; 3) fibrinogen; 4) factor V; 5) platelets.

blood level of tissue plasminogen activator and, consequently, to acceleration of lysis of the fibrin clot [10]. Congenital insufficiency of protein C is accompanied by a high risk of occurrence of thrombotic complications [5]. Another distinguishing feature of protein C is its short circulating half-life ($t_{1/2}$), namely 6-8 h [4]. With the exception of factor VII ($t_{1/2}$ = 6-8 h), the half-life of the remaining vitamin K-dependent blood proteins is several times greater, and amounts to 72-96 h for factor II, 14-20 h for factor IX, and 40-70 h for factor X, which in turn is much less than on average for human blood proteins (10-14 days). The intensity of metabolism of blood clotting proteins is explained by the theory of continuous blood clotting, according to which constant activation and, consequently, utilization of these proteins takes place (the rate of elimination of activated forms of clotting proteins from the blood stream is extremely high, for example, for activated protein C $t_{1/2}$ = 10-15 min [2]). The short half-life of protein C suggests that it is utilized most rapidly and that stimulation or inhibition of blood clotting will affect the rate of activation of protein C.

The aim of this investigation was to study the effect of experimental hyper- and hypocoagulation on the half life of protein C in the rat blood stream.

EXPERIMENTAL METHOD

Protein C was obtained from bovine blood by the method in [12]. The protein was homogeneous on electrophoresis in 7.5% PAG in the presence of SDS, and had a molecular weight of 62 kilodaltons. The protein C was iodinated with Na^{125}I, using iodogen, by the method suggested by the firm "Amersham." Iodogen (20 μl) was introduced into a polyethylene tube and dried. Next, 10 μl of 0.25 M Na-phosphate buffer (pH 7.5), 10 μl of Na^{125}I (40 MBq), and 40 μl of protein C (15 μg) were added to the tube and the contents mixed for 30 min. The iodinated protein was separated from the excess of ^{125}I by gel-filtration on a column (0.9 × 12 cm) with Sephadex G-25. The specific radioactivity of the preparation was 29 μCi/μg protein. To determine the half-elimination time of ^{125}I-protein C from the rat blood stream, the labeled protein was injected in a volume of 1 ml, with total radioactivity of 12 μCi, through the jugular vein into animals (n = 8) weighing 200-250 g. At various time inter-

vals 0.2 ml of blood was taken from the animals into 0.1 ml of 3.8% citrate solution and the radioactivity of the samples was determined in a "Riagamma 1271" γ-counter (LKB, Sweden). Experimental hypocoagulation was induced by a single injection of 20 U heparin in 1 ml of physiological saline into rats (n = 5). Hypercoagulation was induced by injecting thromboplastin solution with activity of 18-20 sec into animals (n = 8) throughout the duration of the experiment at the rate of 0.15 ml/min by means of a peristaltic pump. The effect of heparin and thromboplastin on clearance of ^{125}I-protein C was determined 1 h after injection of the label into the animals. Over a period of 30 min blood was taken from the rat every 5 min, after which heparin was injected intravenously or infusion of thromboplastin began, and blood samples were taken for a further period of 40 min. The development of hypercoagulation was monitored in 24 rats. The activated partial thromboplastin time (APTT) and the thrombin time were determined by the usual methods, activity of factor V by a one-stage method using plasma deficient in factor V ("Dade," Switzerland), the fibrinogen concentration as in [9], soluble fibrin as in [11], and the platelet count was determined in a stained blood film. All experiments with animals were conducted under urethane anesthesia (900 mg/kg).

EXPERIMENTAL RESULTS

After intravenous injection of ^{125}I-protein C into the rats the half-elimination time of the label was 2.3 h (Fig. 1). Intravenous injection of heparin lengthened $t_{1/2}$ of protein C to 6.5 h (Fig. 2a). The possible explanation of this is that, first, heparin accelerated inhibition of thrombin by antithrombin III and, second, it inhibits thrombin generation, thereby also accelerating inactivation of factors IXa and Xa by antithrombin III. Lowering the concentration of thrombin formed in the blood probably leads to a decrease in the activation of protein C and, consequently, to lengthening of its half-elimination time from the blood stream.

Intravenous injection of thromboplastin into the rats led to an increase in the rate of elimination of the label from the blood stream during infusion up to values corresponding to $t_{1/2}$ = 45 min (Fig. 2b). The study of parameters of hemostasis during infusion of thromboplastin yielded evidence of thrombin generation in the course of the experiment (Fig. 3). Thus the platelet count fell by half before the second minute of infusion, and fell to 14% by the 30th minute. The concentration of soluble fibrin was more than doubled (up to 223%) by the second minute, further evidence of the presence of thrombin generation. The fibrinogen concentration fell after 30 min to 39% and the factor V concentration to 21%. Under the experimental conditions (the animals were anesthetized) thrombin did not cause excitation of the anticlotting system or reflex release of heparin [1], for the thrombin time was virtually unchanged during 20 min of infusion. Lengthening of the thrombin time (to 179% by the 30th minute) was probably due to lowering of the fibrinogen level. Reduction of the factor V concentration was the result of its inactivation by protein C, whereas lengthening of APTT (to 340% by the 30th minute) was connected with a fall in the concentrations of both factor V and fibrinogen.

It can be concluded from the results described above that during infusion of thromboplastin into the animals continuous thrombin generation took place. The increase in the rate of elimination of ^{125}I-protein C from the blood stream reflects its activation by thrombin, since the activated form of protein C is removed from the circulation much more rapidly than the inactive form (the proenzyme), and this leads to a decrease in the concentration of the radioactive label in the animal's blood.

Our results confirm the hypothesis relative to the high reactivity of the protein C system. Further evidence in support of this view also is given by the high affinity of thrombin for thrombomodulin (K_{dis} = 0.48 nM), complex formation with which is a condition of activation of protein C. Activated protein C inhibits the generation of new portions of thrombin and, additionally, thrombin in the form of a complex with thrombomodulin is internalized by endothelial cells [8].

It can be tentatively suggested that the protein C system is the first or one of the first mechanisms limiting thrombin formation.

Thus the half-elimination time of protein C from the rat's blood stream if 2.3 h. Experimental hypocoagulation leads to slowing, whereas hypercoagulation leads to quickening of activation of protein C, which reflects the direct involvement of protein C in the regulation of the liquid state of the blood.

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EFFECT OF CALCITONIN ON MECHANISMS OF URINE FORMATION AND SODIUM EXCRETION IN HORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

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546.33[-02:615.357:577.175.446]-092.9

KEY WORDS: calcitonin; spontaneous hypertension in rats; kidney; urine formation

An important role in the genesis of hypertensive states, especially spontaneous hypertension of rats, is ascribed to disturbances of calcium metabolism [7, 9, 11]. Accordingly, attention has recently been paid to the study of the possible role of calcium-regulating hormones in these disturbances. Considering the importance of the kidneys in physiological regulation of blood pressure and calcium metabolism, studies of the effects of calcium-regulating hormones on renal activity must be of great interest. Calcitonin has been studied the least in this respect. Effects of calcitonin on excretion of electrolytes (Ca, K, Na, P) have been described in fair detail [2, 5, 8], whereas the effect of the hormone on the mechanisms of urine formation has not yet been fully explained, for there have been reports both of its diuretic [1, 5] and of its antidiuretic effects [6, 11]. However, these data were obtained on normotensive animals, and no description of the study of renal effects of calcitonin in hypertensive animals could be found in the accessible literature.

The aim of this investigation was to study the effects of calcitonin on the excretory function of the kidneys under conditions of spontaneous diuresis in normotensive (NR) and spontaneously hypertensive (SHR) rats.

EXPERIMENTAL METHOD

Experiments were carried out on 80 SHR of the Okamoto-Aoki line and 90 NR of the Wistar line, aged 24-26 weeks; all the rats were males weighing 200-250 g and were kept on a permanent diet of food and water. Animals of the experimental groups were given an injection of a Soviet preparation of hog calcitonin (calcitrin) in a dose of 0.6 U/100 g body weight, whereas the control rats received the same volume of physiological saline. To collect the urine, the animals were kept for 6 h in metabolism cages. The volume of urine excreted was measured, glomerular filtration was determined relative to endogenous creatinine, and tubular reabsorption of water was then calculated. The Na concentration in the urine was studied by flame photometry, and

*Deceased.

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