GENERATION OF ANTICOAGULANT AND FIBRINOLYTIC ACTIVITY OF BLOOD AFTER INTRAVENOUS INJECTION OF PROTEIN C_a INTO RATS

A. E. Kogan and S. M. Strukova

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The liquid state of the blood and clotting are controlled by the joint function of clotting and anticlotting systems [1]. Besides the physiological anticlotting system, there is also a system for biochemical control of blood clotting by inhibitors of active clotting factors (thrombin, factors IXa, Xa, XIa, and XIIa), for example, antithrombin III, cofactor II of heparin, and also an inhibitor of the clotting cofactors (factors Va and VIIIa), which is protein C.

Protein C is a vitamin K-dependent protein of the blood clotting system which circulates in the blood stream in the form of a proenzyme, which is converted by the action of thrombin into a serine proteinase [8, 11]. In the body this process takes place with the participation of thrombomodulin, an endothelial membrane protein, which forms an equimolar complex with thrombin and increases the rate of protein C activation by three orders of magnitude [7]. Unlike other known vitamin K-dependent blood procoagulants (prothrombin, factors VII, IX, and X), protein C possesses anticoagulant and profibrinolytic properties. The anticoagulant effect is due to the fact that protein C_a inactivates factors Va and VIIIa and, consequently, it inhibits thrombin generation [14]. Inactivation requires the presence of protein S, which forms a complex with protein C on a phospholipid surface [13]. The profibrinolytic action of protein C is connected with the fact that it raises the tissue plasminogen activator level and thereby accelerates lysis of the clot [6]. There is an inhibitor of protein C_a in the blood, which forms a covalent complex with the active center of the enzyme [12]. Congenital deficiency of anticoagulant proteins C and S is connected with a high risk of thrombotic complications [9]. The regulatory mechanisms of hemostasis controlled by the protein C system have not been adequately studied.

The intensity of the anticoagulant and profibrinolytic action of protein C may vary in different species of mammals. Infusion of protein C_a led to a rapid rise of the tissue plasminogen activator level in dogs [6] but not in monkeys [5]. In cats, an increase in fibrinolytic activity developed slowly and reached its maximum only after 1 h [4]. Lengthening of the clotting time was found in all animals studied with any lowering of the plasma factor V level.

The aim of this investigation was to study the dynamics of the anticoagulant and profibrinolytic action of protein C_a when injected intravenously into rats and to ascertain whether it is possible to use rats in model experiments to study the regulatory functions of the protein C system.

EXPERIMENTAL METHOD

Bovine protein C was obtained from blood by the method in [11]. The protein was homogeneous on electrophoresis in 7.5% polyacrylamide gel in the presence of sodium dodecylsulfate. Its amidase activity against a synthetic chromogenic substrate, namely N-D-phenylalanyl-pipecolylarginine p-nitroanilide (S 2238, "Kabi Diagnostica," Sweden) was 0.21 μmole substrate/min/μmole protein C.

Protein C was activated with bovine α-thrombin with clotting activity of 1500 NIH units/mg protein, obtained by purification of the commercial preparation [2]. The protein C was incubated with thrombin in the ratio of 100 NIH units thrombin to 1 mg protein C in 0.02 M

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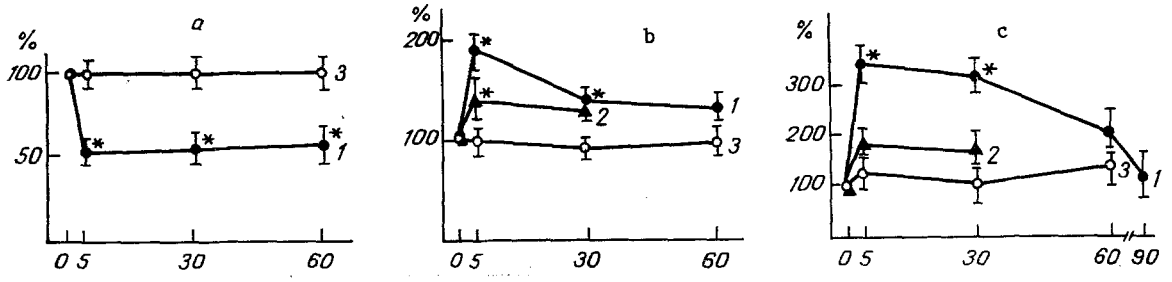

Fig. 1. Changes in activity of factor V (a), in APTT (b), and in activity of plasminogen activators (c) after intravenous injection of various concentrations of protein C_a into rats. 1 and 2) Injection of 0.51 and 0.25 mg protein C_a respectively; 3) injection of physiological saline. *p < 0.05. Abscissa, time (in min); ordinate, % of initial level.

Tris-HCl buffer, pH 7.5, containing 0.1 M NaCl, for 3 h at 37°C. Protein C_a was freed from thrombin by the method in [6]. The protein concentration in preparations of protein C was determined spectrophotometrically, assuming that $D_{280}^{1\%} = 13.7$ [11].

Altogether 39 albino rats weighing 200-250 g were used in the experiment. Under amobarbital anesthesia (70 mg/kg) 1.3 or 2.7 mg of protein C_a/kg body weight was injected in a volume of 1.5 ml into the jugular vein of the rats. The same volume of physiological saline, buffered with Tris-HCl to pH 7.4, was injected into the control animals (n = 9). Blood for investigation was taken from the jugular vein with 2.85% sodium citrate solution in the ratio of 9:1 before injection of the protein C_a and again 5, 30, 60, and 90 min after injection. The activated partial thromboplastin time (APTT) and the thrombin time in the samples were determined by the usual methods, and activity of factor V by a one-stage method with factor V-deficient plasma ("Dade," Switzerland) [10]. Activity of the plasminogen activators was determined by the method in [3].

EXPERIMENTAL RESULTS

The results of two series of experiments to study the effect of different concentrations of protein C_a on the state of the hemostasis system of the rats are given in Fig. 1. It can be seen that 5 min after injection of 0.51 mg of protein C_a the APTT level rose to 190 ± 10.9% ($p < 0.001$), the plasma factor V activity fell to 53 ± 4.9% ($p < 0.001$), and plasminogen activator activity rose to 339 ± 52.8% ($p < 0.001$). A high level of plasminogen activators (305 ± 40.9%; $p < 0.001$), high anticoagulant activity (APTT; 138 ± 4.2%; $p < 0.01$), and reduced factor V activity (54 ± 13.1%; $p < 0.001$) were still found 30 min after injection of the preparation. After 60-90 min APTT and the plasminogen activator activity returned to their initial levels. Meanwhile factor V activity remained low (60 ± 12.2%, p. < 0.001). Reduction of the concentration of injected protein C_a by half did not affect the character of the changes in hemostasis, but weakened the effects. APTT 5 min after injection of 0.25 mg protein C_a was 142 ± 19.2% ($p < 0.01$) and plasminogen activator activity was 184 ± 30.6%.

In all the experiments the thrombin time in the plasma also was determined (it increases on the appearance of heparin in the blood). No significant difference in the thrombin time after injection of protein C_a from the basal level could be recorded (data not given). In our opinion the increase in anticoagulant activity in the plasma was unrelated to the appearance of heparin in the blood stream, but was due to a decrease in factor V activity.

Since the maximal anticoagulant effect was observed as early as 5 min after injection of protein C_a, it was interesting to discover how quickly the hemostasis system reacts to the appearance of this enzyme. In the next series of experiments the state of the hemostasis system was investigated 1 min after injection of protein C_a. An increase in APTT (the most informative test) was found after 1 min to 214 ± 8.9% ($n = 5$, $p < 0.001$). Thus anticoagulant activity reached its maximal value during the first minutes after injection of protein C_a. Since factor V activity did not fall with the passage of time, but APTT gradually returned to normal, it can be tentatively suggested that inactivation of factor V by protein C_a is complete during the first few minutes after injection of protein C_a. Meanwhile activity of the enzyme is evidently blocked by a plasma inhibitor.

It follows from the results described above that the blood clotting system of rats responds actively to injection of protein C_a: APTT is doubled and the plasminogen activator level is raised threefold during the first few minutes. It can be postulated that the anticoagulant reaction manifested as an increase in APTT follows a similar course in rats, dogs, cats, and monkeys. The response of the fibrinolytic system of rats differs from the response of cats and monkeys and is similar to that in dogs, although in rats the effect lasts longer.

We found a decrease of 50% in factor V activity, in agreement with experiments in vitro to study inactivation of human factor Va by protein C_a [14].

The blood clotting system of rats can thus be used as a model with which to study the role of protein C in the system regulating hemostasis.

LITERATURE CITED

1. B. A. Kudryashov, Biological Problems in Regulation of the Liquid State of the Blood and its Clotting [in Russian], Moscow (1975).
2. S. M. Strukova, B. A. Umarova, E. G. Kireeva, et al., Biokhimiya, 43, No. 4, 708 (1978).
3. T. Astrup and S. Mullertz, Arch. Biochem., 40, 346 (1952).
4. M. D. Burdic and R. G. Schaub, Thombos. Res., 45, No. 4, 413 (1987).
5. M. Colucci, J. M. Stassen, and D. Collen, J. Clin, Invest., 74, 200 (1984).
6. P. C. Comp and C. T. Esmon, J. Clin. Invest., 68, No. 5, 1221 (1981).
7. C. T. Esmon and N. L. Esmon, Sem. Thrombos. Hemostas., 10, No. 2, 122 (1984).
8. W. Kisiel, L. H. Ericsson, and E. W. Davie, Biochemistry (Washington), 15, No. 22, 4893 (1976).
9. R. A. Marlar, Sem. Thrombos. Hemostas., 11, No. 4, 387 (1985).
10. M. S. Sirridge and R. Shannon, Laboratory Evaluation of Hemostasis and Thrombosis, Philadelphia (1983).
11. J. Stenflo, J. Biol. Chem., 251, No. 2, 355 (1976).
12. K. Suzuki, J. Nishioka, H. Kusumoto, et al., J. Biochem. (Tokyo), 95, No. 1, 187 (1984).
13. F. J. Walker, Sem. Thrombos. Hemostas., 10, No. 2, 131 (1984).
14. F. J. Walker, P. W. Sexton, and C. T. Esmon, Biochim. Biophys. Acta, 571, No. 2, 333 (1979).

CELLULAR MECHANISMS OF TRANSIENT CONTRACTION OF CORONARY ARTERIAL SMOOTH MUSCLES IN HYPOXIA: ROLE OF INTRACELLULAR Ca^{++}

A. I. Solov'ev, S. A. Bershtein, and O. V. Bazilyuk

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Pacemaker activity of vascular smooth-muscle cells (SMC) is known to be depressed in hypoxia, and their maintained level of tension falls. The basic mechanisms of this phenomenon have been established [1, 4, 10] – a decrease in excitability and calcium conductivity [8] of the sarocolemma of SMC and a decrease in the sensitivity of their contractile proteins to Ca^{++} [2, 6]. In recent years however, evidence has been obtained [3, 9, 11, 13] that SMC of the coronary arteries respond to a fall in the level of oxygenation by a biphasic constrictor-dilator reaction. The mechanisms of transient hypoxic contraction (THC) of the coronary SMC in oxygen deficiency remain largely unexplained. For instance, the sources of Ca^{++}, activating the contractile system of SMC, have not been identified, and this remains an obstacle for the search for pharmacological agents capable of effectively blocking the devel-

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Department of Physiology of the Circulation, A. A. Gogomolets Institute of Physiology, Academy of Sciences of the Ukrainian SSR, Kiev.

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