INTRODUCTION

The hypothalamus–neurohypophyseal system is a center of neurohumoral regulation, whose functions are mediated by regulatory peptides produced by the neurosecretory nuclei of the hypothalamus. Proline-rich hypothalamic neuropeptides discovered by A.A. Galoyan have a wide spectrum of biological activity [1]. One of them, PRP-1, consisting of 15 amino acid residues, has immunomodulating, neuroprotective, antibacterial, and cytokine properties [2]. PRP-1 regulates humoral and cellular immunity, myelopoiesis, and thymocyte differentiation [3, 4].

On the basis of experimental data, Galoyan proposed a hypothesis on the neuroendocrine immune system of the brain, which is important not only for functioning of the hypothalamus–pituitary–adrenal axis but also for the immune system of the brain per se [5].

Studies on the effects of PRP-1 on the hemostasis system revealed its anticoagulation influence on platelet aggregation in plasma in vitro, which suggests that this peptide has a similar effect in the body in vivo. Previous studies on thromboplastic PRP-1 activity revealed its ability to inhibit platelet aggregation in human blood plasma. Therefore, studies on the involvement of this peptide in the regulation of metabolism of the key enzyme of hemocoagulation and the marker of the general coagulation pathway, Xa, seem to be very important.

It has been demonstrated that PRP-1 acts at the stage of activation of factor X (i.e., the formation of factor Xa) when internal and external mechanisms of blood coagulation “combine” after the formation of factor Xa in accordance with the same reactions of the coagulation cascade. It has been shown that factor Xa forms a complex with its inhibitor antithrombin III (AT III); this helped to reveal two forms of factor Xa, Xαα and Xαβ, and a modified form of AT III and to demonstrate the release of native AT III, free factor Xa, and the products of proteolysis of factor Xa.

PRP-1 has a dose-dependent biphasic effect on the formation of a complex of Xa-AT III. At high doses, this process was activated, whereas at low doses, it was inhibited [6]. A new alternative pathway of the metabolism of factor Xa, which probably includes its autolysis, was proposed. In the present study, we examined the effects of PRP-1 on several factors of the hemostasis system in vivo under conditions of the normal functioning of this system.

MATERIALS AND METHODS

The experiments were performed with male albino rats weighing 180–200 g. Ninety intact animals were divided into two groups. The rats in group 1 were injected intravenously with isotonic saline and used as the control. The animals of group 2 (n = 65) were injected with PRP-1 at doses of 1–10 μg/100 g of body weight into the jugular vein. Blood was sampled from the same vein 30 and 60 min after the drug administration using 3.8% sodium citrate as an anticoagulant at a ratio of 9 : 1. The samples were centrifuged at 2000 rpm for 10 min. Blood plasma was used for the following experiments. For studies on hemostasis, we estimated the time of blood coagulation and recalcification [7] and the indices of thrombogenesis and activity of factors of prothrombin complex [8]. The anticoagulation system was studied using the changes in plasma tolerance to heparin, thrombin time, and fibrinogen content [9, 10].

For the experiments, we used thrombin (T6884, Sigma) and thromboplastin (Delat-THR-stb). PRP-1 was synthesized in the Laboratory of Natural Com-
pounds at the Chemistry Faculty of St. Petersburg State University.

Statistical differences between control and experimental groups were calculated using non-parametrical Mann–Whitney U-test. The differences were considered as significant at $p < 0.01$.

RESULTS AND DISCUSSION

The liquid state of the blood is maintained due to dynamic interaction of procoagulant, anticoagulant, and fibrinolytic reactions. The result of this interaction is the coagulation properties of the whole blood, which allow one to estimate its condition.

Studies on the effects of PRP-1 on changes of coagulation were performed in intact rats in vivo. Our data show clear acceleration of blood coagulation, which depended on a dose of the injected drug. At a low dose of 1 $\mu$g/100 g of body weight, we observed a procoagulation effect, viz., a shortening of the time of spontaneous coagulation by 35.5%. An increase in the dose to 2.5 $\mu$g reduced coagulation or did not influence it, indicating the presence of a limit of the acceleration of hemocoagulation and dose-dependence of the PRP-1 effect (Fig. 1a).

Data on the effect of PRP-1 on the time of plasma recalcification demonstrate that these two indices change in one direction. The time of recalcification decreased 30 min after PRP-1 administration at a dose of 1 $\mu$g/100 g of body weight by 44%; this effect was maintained for 60 min. An increase in the PRP-1 dose to 2 $\mu$g accelerated plasma recalcification by 81%, whereas a higher dose of 10 $\mu$g decreased this effect to 51% (Fig. 1b).

Assessment of prothrombin time also supported the multifunctionality and dose-dependence of the PRP-1 effects. A low dose of 1 $\mu$g/100 g of body weight of PRP-1 decreased this index by 31.3%. The strongest increase in prothrombin time by 35.7% was observed after administration of 2.5 $\mu$g of PRP-1, whereas a dose of 10 $\mu$g of PRP-1 did not change this index (Fig. 1c).

Taken together, these data show that at low doses PRP-1 caused a dose-dependent hypercoagulation shift of the coagulation indices. On the basis of these data, we performed an additional experimental series in order to study the effect of PRP-1 on the functional state of the anticoagulation system.

The literature data suggest that acceleration of blood coagulation is related not only to increased activity of factors that stimulate coagulation but also to decreased levels of substances that inhibit coagulation [11].

Our data show that administration of PRP-1 to intact rats was followed by the suppression of the anticoagulation system, which was seen as changes in both anticoagulant and fibrinolytic processes of hemostasis. PRP-1 at a dose of 2 $\mu$g decreased plasma tolerance to heparin by 52% in 30–60 min after its administration (Fig. 2c, 1). An increase in the dose of PRP-1 to 5 $\mu$g further decreased this index to 68% (Fig. 2c, 2). The decrease in the thrombin time was 25% (Fig. 2b, 1), whereas the fibrinogen content increased by 3.5 times compared to the control level (Fig. 2a, 1).