Study of the Mechanism of Hemostatic Effect of Desmopressin

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Intravenous injection of 1 μg/kg desmopressin to rabbits not only accelerated clotting of arterial blood (maximally by 59.16±8.53% after 1 h), but also increased the number of microvesicles containing the integral enzyme 5'-nucleotidase in the arterial blood from the initial level of 36.26±8.08 ncat/liter to a maximum of 99.65±15.8 ncat/liter after 15 min.

Key Words: *desmopressin; microvesicles; blood clotting; hemostasis*

Desmopressin (1-deamino-8-D-arginine-vasopressin; DP) is a long-acting synthetic analog of natural natriuretic hormone. It interacts with type V_2 (but not with type V_1) vasopressin receptors and therefore does not increase blood pressure. Desmopressin injected to volunteers and patients with benign hemophilia A and type 1 von Willebrand disease induces mobilization of factor VIII:C and von Willebrand factor (WF) from depots and 2-3-fold increases their plasma concentrations [7]. Normally WF stored in Weibel—Palade bodies is constitutively secreted by endothelial cells in the form of low-molecular-weight multimers. Agonists induce regulated exocytosis of large multimers [4]. The release of WF from Weibel—Palade bodies of endothelial cells in response to stimulation of type V_2 receptors with DP is mediated by the formation of cyclic AMP [6]. Apart from factor VIII and WF, the same agonist induces the release of tissue plasminogen activator (TPA), a fibrinolysis inductor [4], from endothelial cells. However, not all hemostatic effects of DP can be attributed to WF [5].

Epinephrine produces a similar effect on secretion of WF, factor VIII, and TPA. We showed that activation of blood coagulation after injection of epinephrine to rabbits is accompanied by enhanced release of endothelial and platelet microvesicles (0.05-3.0 μ in diameter) exhibiting a procoagulant effect [1].

Here we studied in detail the mechanism of the hemostatic effect of DP and tried to clear out whether activation of blood clotting under the effect of DP was paralleled by an increase in the number of circulating microvesicles in rabbit plasma.

MATERIALS AND METHODS

Awake rabbits (n=6; 2.9-3.6 kg) were intravenously injected with 1 μg/kg DP (adiuretin, Ferring) in 1.5 ml 0.85% NaCl. A similar dose was used in awake dogs [3]. Control rabbits (n=5) of the same weight were intravenously injected with 1.5 ml 0.85% NaCl.

Blood for the analysis was collected from the femoral artery via Teflon cannulas. Clotting activity was evaluated by the blood clotting time in Bazaron device at 37°C and 100% humidity on paraffin surface [2]. For plasma separation, the blood was stabilized with crystalline heparin (1 mg/ml; Spofa) and centrifuged for 20 min at 960g. The number of plasma microvesicles formed from external cell membranes was evaluated by activity of the marker enzyme 5'-nucleotidase [1], the concentration of extracellular hemoglobin in the plasma repeatedly

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centrifuged for 20 min at 2670*g* was evaluated by absorption at λ=540 nm in quartz microcuvettes in a Spekol 20 spectrophotometer (Zeiss).

Statistical analysis was carried out using Fisher's method.

RESULTS

The hemostatic effect of DP manifested directly after its injection by shortening of the blood clotting time in comparison with the initial level and control (Fig. 1).

Preliminary experiments were carried out for evaluation of microvesicle release into the blood. Heparin-stabilized rabbit plasma with high content of microvesicles was filtered through a Vufs Synthesia ultrafilter with 0.1-0.3-μ pores. Plasma activity of 5'-nucleotidase before filtration was 92.62± 7.31 ncat/liter, after filtration 23.48±3.21 ncat/liter. Hence, 74.7% microvesicles were retained by this filter, while 25.3% particles passed through it. Thus, activity of the integral enzyme of external cell membranes (5'-nucleotidase) is the marker of circulating microvesicles of predominantly this diameter.

Activity of 5'-nucleotidase in heparin-treated arterial blood before DP injection was 36.26±8.08 ncat/liter. It increased directly after DP injection and reached the maximum after 15 min (Fig. 2). Activity started to decrease after 30 and 60 min, but approximately 2-fold surpassed the initial level (Fig. 2).

In parallel with evaluation of the number of microvesicles, we measured plasma content of free hemoglobin.

No appreciable differences in arterial plasma hemoglobin concentrations were detected in rabbits injected with DP and 0.85% NaCl at different terms of the experiment and in different samples. This means that the effect of DP is specifically realized through type V_2 receptors and does not involve erythrocytic membrane, though this membrane can release microvesicles containing hemoglobin and form pores letting it through [1].

Desmopressin induced the release of WF and factor VIII into the blood and increased the number of microvesicles formed by external cell membranes and containing integral 5'-nucleotidase in the blood. However, the dynamics of increase in the number of microvesicles did not completely coincide with the dynamics of blood clotting. The greatest acceleration of blood clotting in our experiments was observed 60 min after DP injection, while the number of microvesicles reached the maximum 15 min after injection and then started to decrease (Fig. 2).

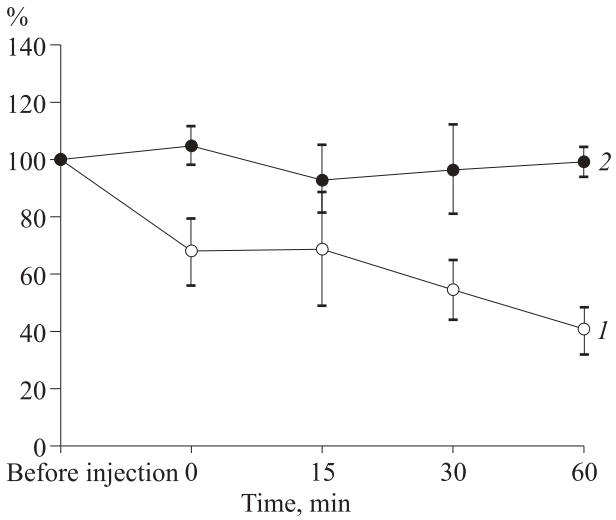

Fig. 1. Changes in blood clotting time in rabbits after intravenous injection of 1 μg/kg DP (*1*) or 1.5 ml 0.85% NaCl solution (*2*).

In experiments on dogs, the maximum increase in WF concentration after DP injection in the same dose was observed after 30 min and persisted for more than 4 h, while the effect of maximum increase in factor VIII concentration lasted for less than 4 h and TPA activity returned to normal as soon as after 60 min [3]. In patients injected with DP, plasma content of platelet microvesicles increased to 127% 1 h postinjection (p<0.05) [5].

Presumably, the dynamics of the number of microvesicles, plasma concentrations of WF, factor VIII, and TPA activity reflected not only the rate of their release into the blood, but also the intensity of their elimination from circulation. Blood clotting velocity is an integral parameter and obviously depends not only on microvesicles, because it continues to increase despite the decrease in their number.

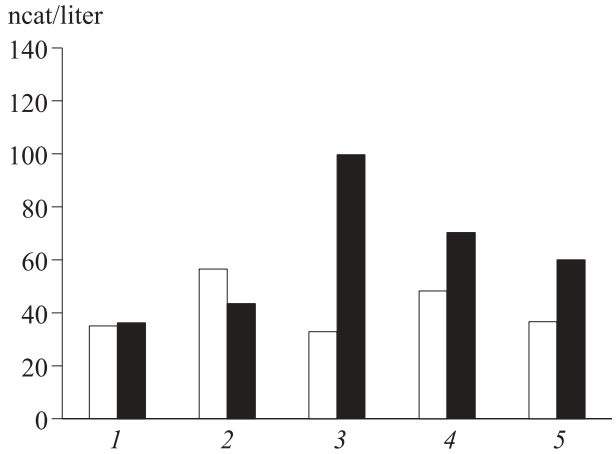

Fig. 2. Number of microvesicles in arterial blood of rabbits after intravenous injection of 1 μg/kg DP. Light bars: 0.85% NaCl; dark bars: DP. *1*) initial number of microvesicles; *2*) at the moment of injection; *3*) after 15 min; *4*) after 30 min; *5*) after 60 min.

Our data indicate that endothelial cells, similarly as platelets, release microvesicles [1]. Separation of microvesicles from the endothelium is a sign of its modification. The microvesiculation process is directly linked with disorders in lipid asymmetry of the external cell membranes with appearance of phosphatidylserine on their surface [8,11]. The data suggest that DP-initiated and scramblase-realized transfer of phosphatidylserine from the inner to the outer leaflet of endotheliocyte membrane and that hypercoagulemia precede microvesiculation and persists after it, thus providing high level of blood coagulation.

It is interesting to evaluate the relationship between regulated secretion of WF and microvesiculation. Modern data indicate that the increase of the cytosol concentration of Ca^{2+} triggered by external signals is a common feature of these processes. However, the antegrade transport of WF from Weibel—Palade bodies (secretory granules of vascular endotheliocytes) is realized by structures different from those involved in the formation of microvesicles. Microvesiculation starts from Ca^{2+}-induced trans-bilayer migration of membrane phospholipids, leading to local loss of the lipid asymmetry. Though all main phospholipid classes are involved in this process, the rate of inward migration of sphingomyelin is significantly lower than the rate of trans-bilayer migration of other (glycero)phospholipids [9,10]. This difference disorders the balance between the bilayers, increases surface tension in the outer leaflet in comparison with the inner one, and leads to external protrusion of the plasma membrane. This can facilitate shedding of microvesicles for leveling the surface tension between the bilayer leaflets. However, impairment of the lipid asymmetry can persist longer than the microvesiculation process. We consider [2] that this asymmetry is an additional cause of blood clotting activation, because it increases procoagulant activity of the endothelial lining of blood vessels because of formation of the tenase and prothrombin complexes of the blood coagulation system on phosphatidylserine clusters.

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