Dear Sir,
It is commonly believed that the morning surge in blood pressure, along with haemostatic changes that promote thrombosis, contributes to cardiovascular events and death at this time of the day. Some studies have shown that aldosterone has circadian rhythmicity and that the major peak of aldosterone occurs during early morning hours (1). It has also been shown that acute systemic infusion of aldosterone in normotensive volunteers results in endothelial dysfunction (2). This observation suggests that even a short-lasting rise in aldosterone level may potentially promote the thrombotic process, by reducing the antithrombotic properties of the endothelium. Therefore, in the present study we wanted to elucidate whether acute infusion of aldosterone could affect experimental thrombosis in rats. The contribution of coagulation and fibrinolytic systems in the mode of aldosterone action was also determined. Furthermore, we investigated the role of the mineralocorticoid receptor in the mechanisms of aldosterone action.

Male Wistar rats (300–350 g) were used in this study. All investigations were carried out at the same time of the day (09:00 a.m.) to minimize any effect of diurnal variation in the haemostatic system and vascular function. Aldosterone (ALDO) (Sigma-Aldrich, Poland; 3, 10 and 30 µg/kg/h) or VEH (0.4% ethanol; 2 ml/kg/h) was infused into the femoral vein 5 minutes (min) before the induction of venous thrombosis and was continued for 1 hour (h) and 5 min (Constant-Rate Infusion Pump, Kwapisz, Poland). Eplerenone (EP), a selective mineralocorticoid receptor antagonist (Pfizer Co., Poland), was administered (p.o., 5% aqueous gummi arabic solution) at a dose of 100 mg/kg 30 min before ALDO (30 µg/kg/h) infusion. Venous thrombosis was performed by ligation of the vena cava (3), following pentobarbital anesthesia (Vetbutal, Biowet, Poland, 45 mg/kg i.p.). After 1 h, blood samples were drawn from the right ventricle of the heart prior to thrombus removal. The blood was mixed with 3.13% sodium citrate (Sigma-Aldrich, Poland) in a volume ratio of 9:1 and centrifuged for 20 min at 3,500 x g, at 4°C, and then plasma was deep-frozen (-70°C). The venous thrombus was carefully collected from the vein and weighed. Serum aldosterone concentration was measured by the RIA procedure (DPC, Poland). Plasminogen activator inhibitor (PAI-I), tissue plasminogen activator (t-PA), thrombin activatable fibrinolysis inhibitor (TAFI) and tissue factor (TF) plasma levels

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Acute aldosterone infusion enhances thrombosis development in normotensive rats

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Figure 1: The effect of aldosterone (ALDO) and ALDO with eplerenone (EP) on venous thrombus formation and haemostatic parameters. Data are expressed as mean ± SEM and %. Differences were analysed by Mann-Whitney U test. P-values less than 0.05 were considered statistically significant. The lack of thrombus was defined as 0 mg. The incidence of thrombosis was compared between the groups using the exact Fisher test. ** p<0.01, *** p<0.001 vs. VEH; # p <0.05, ## p<0.01, ### p<0.001 vs. ALDO; n= 8-15.

were measured by enzyme immunoassays (Assypro, USA) in a microtiter plate using a Titertek Twin-Reader (Flow Laboratories) according to manufacturer’s directions. Potassium concentration was measured in urine samples from urinary bladder (Bayer Diagnostic 348 Analyzer). Procedures involving animals and their care were conducted in conformity with the institutional guidelines (4).

We observed that one-hour infusion of ALDO 30 µg/kg/h resulted in a significant rise in venous thrombus weight and in a markedly elevated incidence of venous thrombosis (Fig. 1). ALDO at doses of 3 and 10 µg/kg/h did not change significantly thrombus weight in comparison to VEH. It should be mentioned that ALDO 30 µg/kg/h resulted in a two-fold rise in serum aldosterone level in comparison to the VEH group (data not shown). This is in agreement with observations that concentration of this hormone may reach from 3 to even 20 times the normal level during circadian rhythmicity, hypertension, chronic and acute heart failure (1, 5–7). To investigate the mechanism by which ALDO promotes venous thrombosis we used EP to block the mineralocorticoid receptor. EP administered together with ALDO 30 µg/kg/h prevented the increase in thrombus formation (Fig. 1). We also observed that the increase in thrombus weight in the ALDO-treated group was paralleled by a marked increase in PAI-1 and decrease in t-PA plasma levels, as compared to VEH-treated rats (Fig. 1). Significant rises in TF and TAFI plasma levels were detected. Pretreatment with EP reversed the decrease in t-PA and the increase in TAFI plasma levels and markedly blunted the increase in PAI-1 and TF plasma levels (Fig. 1). An increase in urinary potassium concentration for ALDO 30 µg/kg/h (135 ± 4 mM vs. VEH 57 ± 3 mM; p<0.01, n=8) was also noted. Pretreatment with EP significantly reduced urinary potassium concentration in comparison to ALDO 30 µg/kg/h (77 ± 4 mM vs. 135 ± 4 mM; p<0.01, n=4).

This is the first experimental indication of acute ALDO infusion-enhanced thrombus formation following stenosis-related vascular damage in rats. Some experimental data have demonstrated that ALDO upregulates PAI-I synthesis (8). We also demonstrate that acute ALDO infusion rises PAI-1 plasma level; moreover, high TAFI level implies enlarged thrombin generation during ALDO infusion.

Thus, our study provides direct evidence that acute infusion of aldosterone results in impairment of the fibrinolytic process and activation of coagulation cascade, consequently leading to augmentation of stasis-induced venous thrombosis formation. The prothrombotic effect of aldosterone was mediated by the mineralocorticoid receptor. It also confirms the possibility that short lasting changes in aldosterone level might contribute to the cardiovascular events. Previously, we have demonstrated that angiotensin II and angiotensin IV possess prothrombotic activity in rats (3, 9). Our present study documents novel properties of aldosterone, extending function of this hormone to the mediation of hemostasis.

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References