# Release of tissue-type plasminogen activator (t-PA) in the splanchnic circulation of the anaesthetised pig during high sympathetic tone.

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**Abstract**

Background: There is a substantial local release of tissue-type plasminogen activator (t-PA) in the splanchnic vascular bed, and this release is increased at high sympathetic tone. Coronary t-PA release is also significant, and this release increases during cardiac nerve stimulation and during reperfusion after 10 min of local myocardial ischemia. However, by repeated cycles of myocardial ischemia/reperfusion coronary t-PA release progressively declines.

Objectives: Accordingly, we hypothesised that splanchnic t-PA release might decrease after an initial peak during maintained and long-lasting sympathetic stimulation.

Methods: In 6 anaesthetised pigs sympathetic tone was augmented by bleeding (20-25 mL/kg over 30 minutes). During the subsequent 2 hours period portal vein (draining the splanchnic vasular bed) - and arterial blood samples were drawn every 20 min and portal vein blood flow was recorded continuously in order to estimate t-PA release in the splanchnic vascular bed.

Results: Relatively stable haemodynamic conditions were obtained after bleeding with mean arterial blood pressure at 50 to 65 mmHg and a portal vein flow at about the 50% of baseline value. Net splanchnic t-PA release rose to a peak 40 min after bleeding, but subsequently declined towards baseline values. Arterial t-PA activity rose after the bleeding period and to a peak value at end of the observation period.

Conclusions: Net splanchnic t-PA release increased only transiently during the period with increased sympathetic stimulation, whereas the arterial t-PA level remained elevated. During a strong and longlasting sympathetic stimulation the lack of a continuously augmented splanchnic t-PA release might increase the risk for intravenous splanchnic thrombosis.

Key words: Hypovolaemia; Splanchnic circulation; Sympathetic stimulation; Tissue-type plasminogen activator release

## Introduction

Both a constitutive and a regulated release of the fibrinolytic enzyme, tissue-type plasminogen activator (t-PA), have been demonstrated in various vascular beds. In humans it has been shown that mental stress induces a regulated release of t-PA both from the forearm and the cerebral vascular beds [1, 2]. In experimental animal models activation of the sympathetic nervous system has been shown to induce a regulated t-PA release both from the splanchnic- and coronary vascular beds [4, 5]. However, when cardiac sympathetic tone was repeatedly increased by brief periods of coronary occlusion and reperfusion, net cardiac t-PA release declined progressively [6].

Both the constitutive and the regulated t-PA releases are substantially higher in the splanchnic- than in other vascular beds [1-5]. Therefore, fluctuations in net splanchnic t-PA release may significantly affect whole-body fibrinolytic homeostasis, but it has not been examined if *e.g.* a severe and long-lasting period of sympathetic stimulation results in a progressive decline in net splanchnic t-PA release. As it recently has been shown that coronary t-PA release declines progressively by repeated brief cycles of ischaemia and reperfusion [6], we hypothesised that t-PA release to the splanchnic vascular bed might subsequently decline by a strong and long-lasting sympathetic stimulation, although Seeman-Lodding and co-workers [5] found that splanchnic t-PA release increased progressively for at least 10 min during sympathetic stimulation. However, in their study the sympathetic stimulation was modest, and they did not examine the effect of a stronger or more long-lasting sympathetic stimulation.

Accordingly, in the present study we tested the hypothesis that long-lasting sympathetic activation might cause progressive diminution of net splanchnic t-PA release. We increased the general sympathetic tone in anaesthetised pigs by controlled bleeding (20-25 mL/kg over 30 minutes) and sampled arterial blood and portal venous blood (draining the splanchnic vasular bed) as well as continuously recorded portal vein blood flow in order to estimate splanchnic t-PA release. A relatively stable haemodynamic state was obtained and the t-PA release to the splanchnic circulation could be determined at intervals for two hours after the bleeding.

## Materials and methods

*Animals*

Twelve animals were exposed to the bleeding protocol, but 6 animals were excluded; two because they died during the bleeding period, three because of hypotension (mean arterial blood pressure below 50 mmHg) and one because of hypoglycaemia, leaving 6 male pigs, weighing between 36.0 and 48.7 kg for the final analysis. All animals were acclimatised for a few days before the start of the experiment and fasted overnight with free access to water prior to anaesthesia. This study was approved by the Ethical Committee for Animal Research at the University of Gothenburg , Sweden, and conducted in accordance with the “Guide for the Care and Use of Laboratory Animals” published by the US National Institute of Health (NIH publication No 85-23, revised 1985).

*Anaesthesia*

The pigs were sedated by an intramuscular injection of 5 mg/kg midazolan (Dormicum 5 mg/mL, Roche, Basel, Switzerland) in combination with ketaminol 10 mg/kg (Ketamin 100 mg/mL, Intervet, Danderyd, Sweden). Twenty minutes later an intravenous infusion of 2-5 mg/kg propofol (Diprivan 10 mg/mL, Zeneca Ltd, Macclesfield Cheshire, UK) was started. To get a stabile anaesthesia with a responding sympathetic nervous system, α-choloralose (100 mg/kg, 90%, AldrichChemie, Steinheim, Germany) was infused as an intravenous bolus injection during deep propofol anaesthesia, followed by a continuous iv infusion of 25-50 mg/(kg x h). The pigs were intubated and ventilated (Servo Ventilator 900C, Siemens Elema, Solna, Sweden) with room air. The respiratory rate was held constant at 15 cycles/min. Before the experimental interventions and during the experiment, the blood gases and pH were adjusted to physiological levels by regulating the tidal volume. Body temperature was kept constant throughout the experiments at 38-40° C by covering the animals and by external heating.

*Surgical preparation*

An ear vein was cannulated for infusions of anaesthetics and a Ringer solution; 5 mL/(kg x h) (Pharmacia AB, Stockholm, Sweden). Through polyethylene catheters in the left saphenous and femoral arteries, blood pressure was record by a pressure transducer (Peter von

Berg Medizintechnik GmbH, Kirsheseeon/Eglharting, Germany) and arterial blood sampled for analyses of lactate, glucose, haematocrit, blood gases and pH. The urinary bladder was exposed through a midline incision in the lower abdomen and urine drained by gravity through a Foley catheter Ch 12 (Willy Rüsch AG, Kernen, Germany). Through an upper, midline, abdominal incision, a 14 mm ultrasonic transit time flow probe (T206 Transonic System Inc., Ithaca, NY, USA) was placed around the portal vein. Venous blood from the splanchnic circulation was intermittently obtained through a thin central venous catheter; 1.6 mm o.d. (Secalon Seldy, Ohmeda, Swindon, UK), advanced distally through a small splenic vein into vena porta. A 2.5 mm ultrasonic transit time flow probe (T206 Transonic System Inc., Ithaca, NY, USA) was placed around the right femoral artery. Heart rate was monitored using an electrocardiogram (ECG) obtained from needle electrodes placed subcutaneously at locations corresponding to the precordial unipolar leads V1 and V3 in man, and body temperature was recorded by an esophageal thermistor (AstraZeneca, Mölndal, Sweden).

*Blood sampling and biochemical analyses*

Blood was sampled from the left saphenous artery and portal vein in syringes containing 1/10 0.45 mol/L sodium citrate buffer, pH 4.3, and a mixture of EGTA and glutathione 20 for determination of t-PA and noradrenaline (NA), respectively. Samples were centrifuged within 1-2 minutes. Centrifugation was performed at 5000 x g for 10 min at 4°C. Plasma was subsequently frozen and stored at -70°C until analysed. Plasma t-PA activity was determined with a spectrophotometric parabolic rate assay (SpectrolyseTM/fibrin tPA, catalogue No 101101, Biopool AB) [10]. This assay has been shown to be specific for t-PA in porcine plasma [3]. Samples from each experimental animal were analysed on one single microtiter plate. All samples were analysed in duplicate and mean intra-assay coefficients of variation (CV) was 3.5%. Noradrenaline plasma concentration was determined by plasma liquid chromatography with electrochemical detection [11]. Net release of t-PA and NA to the splanchnic vascular bed was calculated as the veno-arterial difference multiplied by portal plasma flow per unit of time.

*Experimental protocol*

A stabilisation period of 1.5 hour after finishing the surgical preparation was allowed. The animals served as a placebo group in another study and were therefore subjected to a continuous infusion of mannitol 2.5% and glucose 2.5% (0.2 mL/kg) throughout the experiment. During a 30 min period after stabilisation, the animals were bled (20-25 mL/kg) to induce sympathetic stimulation. The speed and amount of blood drainage was adjusted to avoid very abrupt haemodynamic changes. During the subsequent 2 hours observation period, as well as during the previous stabilisation period, haemodynamic signals, ECG and body temperature were measured continuously by a custom made computer program (Pharmlab, AstraZeneca R&D Mölndal, Sweden) and arterial- and portal vein blood was sampled at predetermined time points for biochemical analysis and determinations of pH, blood gases and haematocrit.

*Statistical analysis*

Values are mean ± SEM. Analysis of variance was used to test changes by time (either Tukey's test or Anova on Ranks). Differences between arterial and portal vein t-PA and NA activity and the various haemodynamic variables were examined by Wilcoxon's paired test. $P<0.05$ was regarded as statistically significant.

**Results**

Arterial- and portal venous t-PA activity remained constant during the stabilisation period amounting to 6.4 ± 0.5 and 9.6 ± 1.9 U/mL, respectively (difference: $p< 0.001$)(Fig 1). During these baseline conditions there was a significant net release of t-PA across the splanchnic vascular bed, being on average 2112 ± 444 U/min ($p< 0.01$) (Fig 2). Arterial- and portal venous NA concentrations remained constant during the stabilisation period 0.7-1.0 and 1.3-1.7 pg/mL, respectively (Fig 3), as well as splanchnic vascular bed NA release (Fig 4).

Before bleeding, mean arterial blood pressure and heart rate remained stable between 122 and 124 mmHg and between 117 and 119 beats/min, respectively (Fig 5). Both portal venous blood flow (Fig 6) and femoral arterial blood flow decreased slightly during the stabilisation period. However, arterial- and portal vein pH, pO2, pCO2 , oxygen saturation,

haematocrit, lactate and plasma glucose all remained constant during the stabilisation period (data not shown).

*Effects of bleeding*

Portal vein t-PA activity more than doubled shortly after bleeding, but subsequently declined (Fig 1). During the last hour of the observation period, portal vein t-PA activity was still higher than baseline values, but this difference was not statistically significant. Concomitantly, arterial t-PA activity rose from 6.7 1.9 U/mL just before the bleeding period, to 11.7 1.7 U/mL at the very end of the observation period (Fig 1). Splanchnic t-PA net release rose to a peak immediately after the bleeding period, but subsequently declined rapidly towards baseline values (Fig 2).

Both arterial- and portal vein NA concentrations rose significantly, from baseline values less than 2 pg/mL, by the bleeding, and continued to rise progressively to about 25 pg/mL at the end of the observation period (Fig 3). The average portal venous concentration was higher than the average arterial concentration at all points of measurements. Splanchnic NA release rose progressively from an average basal release of 430 153 pg/mL to a maximal value 100 min after the bleeding period of 1450 369 pg/mL (Fig 4).

Marked haemodynamic changes were induced by the bleeding; mean arterial blood pressure declined from about 125 mmHg to 65 mmHg, but remained relatively stable during the observation period at values between 65 and 50 mmHg, whereas heart rate rose from about 120 beats/min before the bleeding period to about 230 beats/min (Fig 5). Portal venous blood pressure did not decline significantly in response to bleeding, but portal vein blood flow declined to about one half of the control value (Fig 6), and femoral arterial blood flow by about two thirds (data not shown).

Arterial and portal vein pH, haematocrit, arterial and portal vein pO2 and arterial oxygen saturation all remained unaltered by bleeding and during the subsequent observation period (data not shown). However, portal vein oxygen saturation declined from 61.4 ± 2.3 to 24.1 ± 3.2 % ($p<0.01$) after bleeding, and portal vein pCO2 rose from 7.52 ± 0.31 to 9.49 ± 0.39 kPa ($p<0.01$).

**Discussion**

In the present study we confirm the findings by Seeman-Lodding and co-workers [5] of an increase in splanchnic t-PA release in response to increased sympathetic tone. However, we also show that this t-PA release, after an initial peak, subsequently declines at maintained sympathetic stimulation. When Seeman-Lodding and coworkers [5] increased sympathetic tone by elevating positive end-expiratory pressure (PEEP) in anaesthetised pigs, they ended the procedure after 10 min. Arterial- and portal vein t-PA activity rose by 24 and 52 %, respectively, in their study, whereas we in the present study found greater increments of 65 and 150 %, respectively. We also verified that a high sympathetic tone was maintained in our study throughout the 2 hours observation period as arterial- and portal vein noradrenaline concentration progressively rose, as did also splanchnic noradrenaline release.

The most likely explanation for the only transient increase in splanchnic t-PA release despite maintained high sympathetic tone may be exhaustion of t-PA stores. Recently Aspelin and co-workers [6] found that cardiac stores of t-PA apparently also could be exhausted. In their study coronary venous t-PA release progressively decreased towards baseline values when cycles of 10 min of local myocardial ischaemia followed by 30 min of reperfusion, was repeated a total of 4 times. Hypercoagulability has been demonstrated during sympathetic stimulation [7,8]. By long-lasting and severe sympathetic stimulation this hypercoagulability may, however, not be effectively counteracted because of the transient nature of the increase in plasma t-PA release. Accordingly, the risk of intravascular thrombosis might be expected to rise. Nevertheless, the occurrence of thrombosis in the extrahepatic portal vein is relatively uncommon [12] and usually related to severe intraabdominal sepsis, states of hypercoagulability as well as blood flow stasis due to either hepatic cirrhosis or extrinsic vascular compression by *e.g.* a tumor. When portal vein thrombosis occurs, it may lead to intestinal ischaemia and infarction due to retrograde propagation of the clot into venous tributaries within the bowel wall. A high percentage of patients with portal vein thrombosis also have a high incidence of autonomic dysfunction [13].

In the present study we found not only changes in splanchnic t-PA release during the long-lasting sympathetic stimulation, but in addition an increase in arterial t-PA activity. This increase was detected by the first measurement after the bleeding period and remained relatively stable throughout the observation period, but reached the level of statistical significance first at the end of this period. Consequently, this increase cannot be explained just by increased splanchnic t-PA release. This is in line with data from the study by Seeman-Lodding and co-workers using 10 min PEEP [5]. In their study, t-PA turnover in the pre-portal organs and in the liver were determined selectively. The results showed that the increase in systemic t-PA activity was not explained by the increase in splanchnic t-PA release as t-PA activity in the hepatic vein remained unchanged, *i.e.* PEEP-induced splanchnic t-PA release was neutralised by a concomitant increase in hepatic uptake of t-PA [5]. Furthermore, PEEP induced only a modest increase in coronary t-PA release, and pulmonary t-PA release remained unchanged [5]. Thus, the source of the systemic t-PA response to sympathetic activation remains to be elucidated. One potential candidate may be the skeletal muscles [1, 17].

The significance of the present data for humans is uncertain, particularly because the present study was performed in anaesthetised pigs exposed to extensive surgery and a profound and long-lasting sympathetic stimulation due to the hypoveolemia of severe haemorrhage. Future research is therefore necessary to reveal if splanchnic t- PA release decreases also in humans exposed to prolonged and severe sympathetic stimulation and if arterial t-PA activity eventually increases with augmented sympathetic stimulation.

In conclusion the present study shows a transient splanchnic t-PA release during a continuous sympathetic stimulation caused by the hypovolaemia of bleeding. It is suggested that this non-sustained t-PA release at high sympathetic tone might put the body at risk for intravascular thrombosis. However, this effect appears to be counteracted by a slow increase in the level of arterial blood t-PA, most likely due to reduced t-PA metabolization in the liver as a consequence of reduced hepatic inflow and/or an effect on skeletal muscles.

**Acknowledgement**

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**Figures**

Fig. 1 Plasma t-PA activity (U/mL) in arterial and portal vein blood. Note progressive decline in portal vein t-PA concentration after bleeding and a significant increase in arterial t-PA activity at the end of the observation period. Values are means ± SEM, ***** $p<0.05$

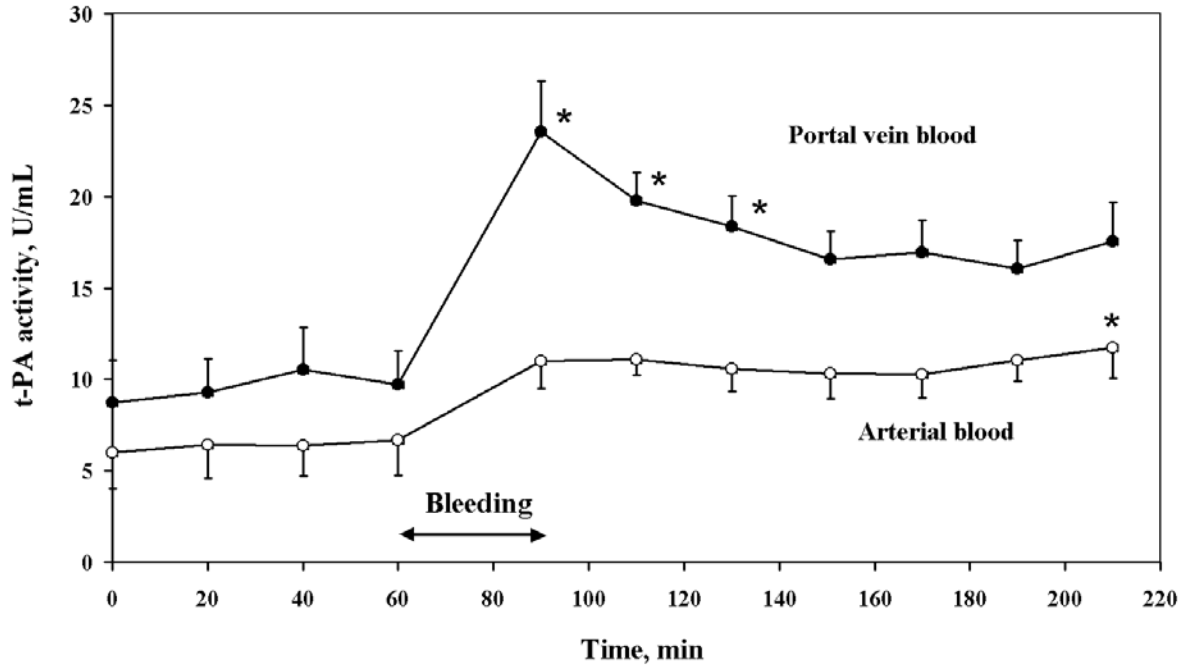


Fig. 2 Splanchnic net release of t-PA activity (U/min). Note progressive decline in t-PA release rates after bleeding throughout the observation period.
Values are means ± SEM , ***** $p<0.05$.

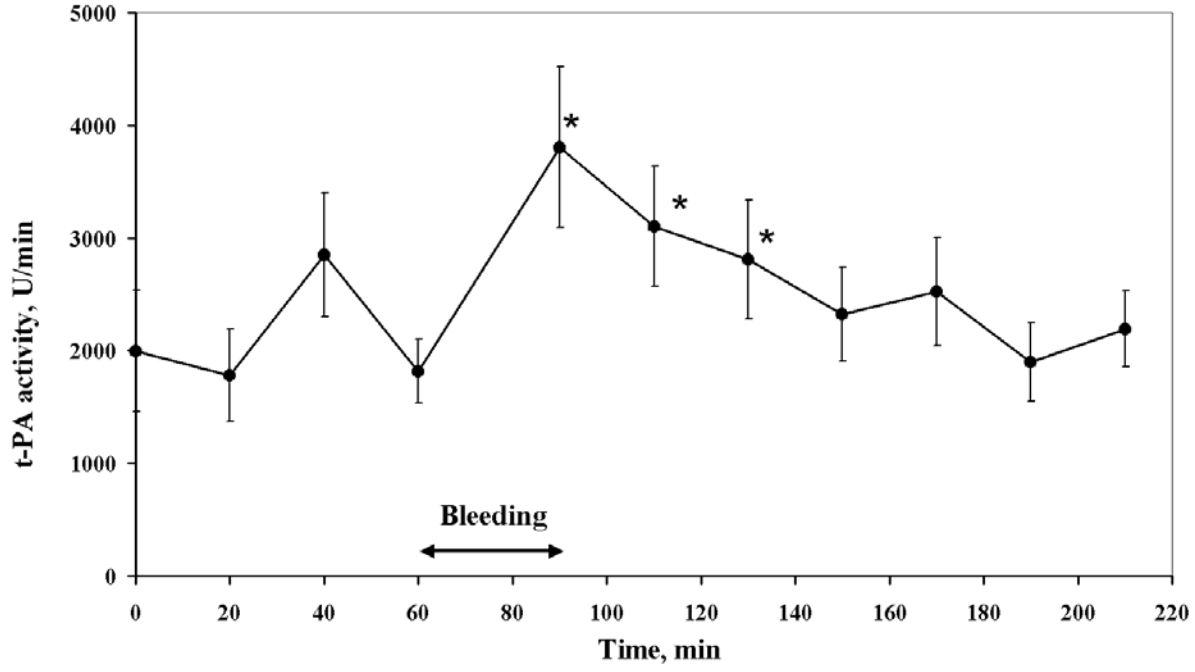

Fig. 3 Plasma levels of NA in arterial and portal vein blood (pg/mL). Note progressive and significant increase for both variables after bleeding throughout the observation period.

Values are means ± SEM , ***** $p<0.05$

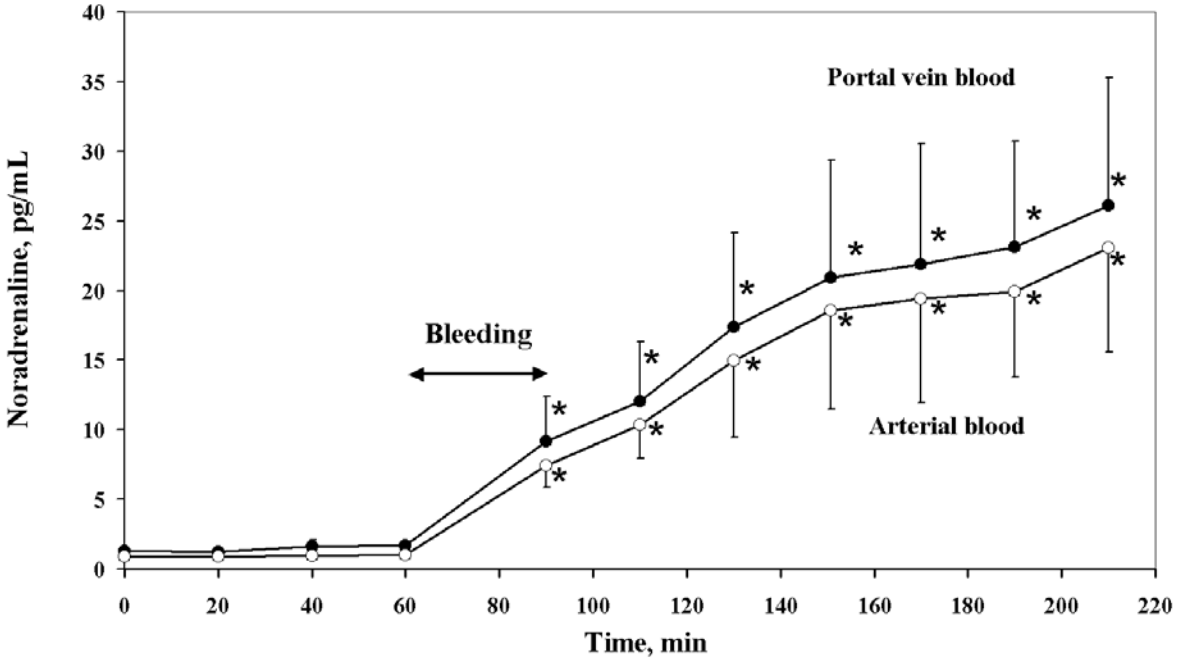


Fig. 4 Splanchnic net release of NA (pg/min). Note progressive increase after bleeding throughout the observation period, and a significantly increased release rate at second to last time point. Values are means ± SEM , ***** $p<0.05$

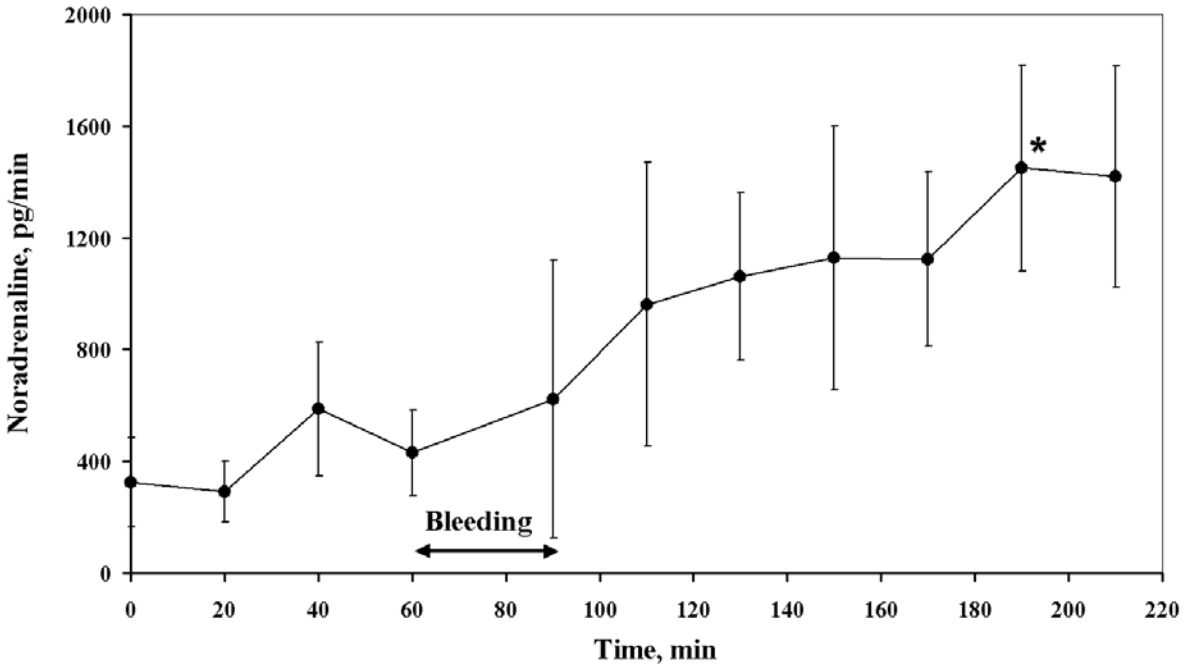

Fig. 5 Heart rate and Mean arterial pressure (beats/min and mmHg). Note relatively stable values after the bleeding period throughout the observation period.
Values are means ± SEM , * $p<0.05$

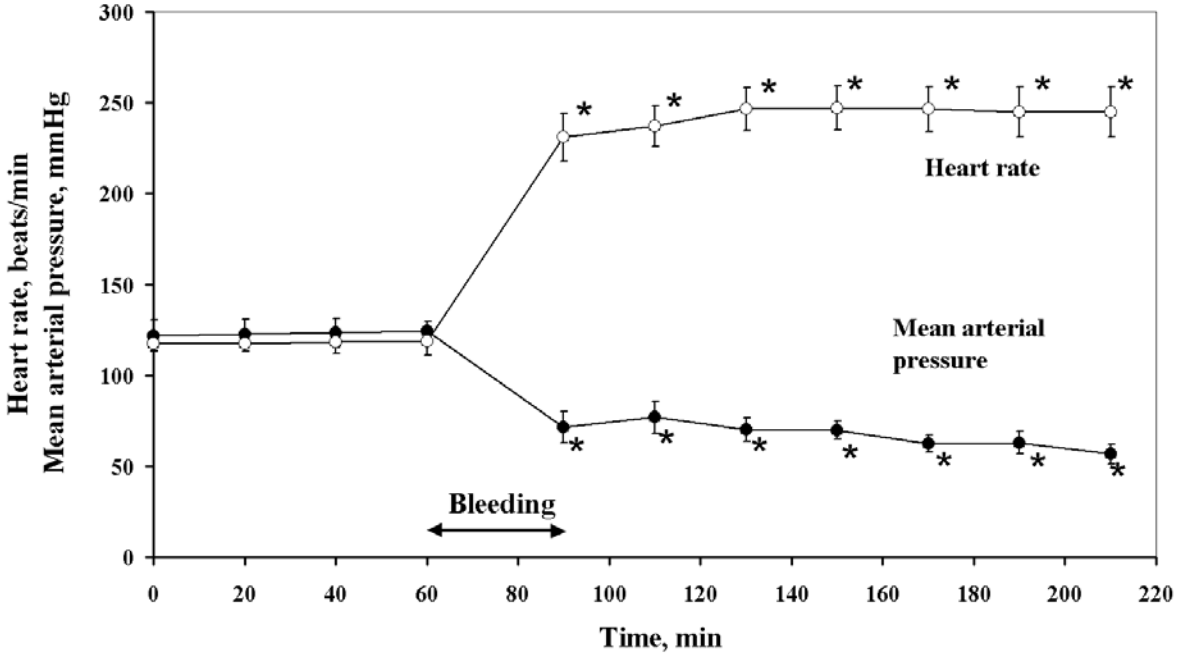


Fig. 6 Portal vein blood flow (mL/min). Values are means ± SEM, * $p<0.05$