Effect of blood pressure on plasma volume loss in the rat under increased permeability

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Abstract

Objective: To evaluate the effects of noradrenalin-induced increase and metoprolol/clonidine-induced decrease in blood pressure on the plasma volume expansion of 5% albumin under increased permeability.

Design: Prospective randomised laboratory study.

Subject: Forty-five adult male Sprague-Dawley rats.

Interventions: Permeability was increased via an anaphylactic reaction by injection of 0.5 ml dextran 70. One hour later, volume expansion with 15 ml/kg of 5% albumin was given for 15 min. Plasma volume was measured just before and 2.5 hours after the albumin infusion (^{125}I-albumin tracer technique). The study included a control group, a noradrenalin group and a metoprolol/clonidine group (n=10 in each group). The vasoactive treatment started after albumin infusion and continued throughout the experiment. Plasma volume was also measured in a separate group without albumin infusion, to evaluate the effect of noradrenalin-induced increase in blood pressure under hypovolemia (n=9). CVP was measured to estimate the venous pressure effect of noradrenalin (n=6).

Results: The remaining increase in plasma volume 2.5 h after infusion of 15 ml/kg of albumin was 11.8 ± 3.6 ml/kg in the control group compared with 0.5 ± 6.3 ml/kg in the noradrenalin group ($p < 0.001$) and with 12.5 ± 4.9 ml/kg in the metoprolol/clonidine group (ns). The reduction in plasma volume by noradrenalin infusion was much smaller in the group not given albumin, or 3.5 ± 3.0 ml/kg.

Conclusion: Noradrenalin infusion increases the loss of plasma volume during a state of increased permeability which, according to the 2-pore theory of transvascular fluid exchange, may be explained by increased hydrostatic capillary pressure. The loss was less pronounced in hypovolemia.

Keywords: Albumin, Arterial pressure, Noradrenalin, Plasma volume expansion, Vascular permeability, Vasopressors

Introduction.

Systemic Inflammatory Response Syndrome (SIRS), caused for example by infection, trauma, burns, pancreatitis, extensive surgery or immunotherapy, is common in the ICU [1, 2]. Patients with SIRS suffer from increased microvascular permeability [3-6], which may result in hypovolemia, in turn leading to reduced venous return and cardiac output, as well as generalized vasoconstriction due to activation of the baroreceptor reflex [7]. Altogether, these alterations predispose the patient to the development of tissue ischaemia, reperfusion injury and organ failure [8-10]. One goal in the treatment of these patients, therefore, is to restore and maintain an adequate circulating blood volume. This means that there is a need for repeated fluid administration [10, 11].

Fluid and proteins are supposed to be transported mainly across the capillary membrane by passive mechanisms as described by the so-called 2-pore theory of transvascular exchange [12]. According to this theory, fluid and small solutes pass the capillary membrane through all pores along the entire microvascular bed, whereas proteins pass the capillary membrane through the less common larger pores (by a factor of 10–30 $\times 10^3$ times) of the venous side of the capillary network and in the venules. As the oncotic pressure gradient across the large pores is significantly reduced, the transcapillary/transvenular hydrostatic pressure is the major force responsible for fluid flow through these pores The proteins are lost passively to the interstitium, mainly via convection when following the large-pore fluid stream, while the diffusion effect is of less significance. This hypothesis also also means that the protein loss is dependent on both the number of large pores (large pore permeability) and the hydrostatic capillary pressure. As

the hydrostatic capillary pressure increases with increase in arterial pressure, it can be expected that an increase in arterial pressure will increase the loss of plasma fluid and proteins to the interstitium via the large pores. This would occur especially under conditions of depressed autoregulation, which is common during sepsis and SIRS or following trauma, as there will be a larger variation in hydrostatic capillary pressure with variation in arterial pressure [13]. This hypothesis offers a therapeutic strategy to reduce the need for blood volume substitution in critically ill patients by decreasing an unnecessarily high arterial pressure and, if confirmed, the results would also support the 2-pore theory for transvascular exchange of fluid and proteins.

Based on these considerations, the aim of the present study was to evaluate the extent to which arterial pressure influences the plasma volume-expanding effect of albumin during a state of increased permeability. Noradrenalin was given with the purpose of increasing arterial pressure, while a mixture of the anti-hypertensive substances metoprolol and clonidine was given with the purpose of reducing arterial pressure. Permeability was increased in a standardized manner by injection of a small dose of dextran, which is known to cause an anaphylactic reaction with plasma leakage in the rat [14,15].

Methods

Materials and anaesthesia

The study was approved by the local ethics committee for animal research, and the animals were treated in accordance with the Guidelines of the National Institutes of Health for Care and Use of Laboratory Animals. Adult male Sprague-Dawley rats (n = 45), weighing 350 ± 12 g were used. Anaesthesia was induced by placing the animals in a covered glass container with a continuous supply of isoflurane (Forene; Abbot, Stockholm). The animals were tracheostomized under isoflurane anaesthesia via a facemask and connected to a ventilator

(Ugo Basile; Biological Research Apparatus, Comerio, Italy) using a PEEP of 2.5 cm H_2O. Thereafter, anaesthesia was maintained by administration of 1.5–1.8% isoflurane through the tracheal cannula. Body core temperature measured rectally was maintained at 37.1–37.2ºC via a feedback controlled heating pad. End-tidal PCO_2 was monitored continuously and kept between 4.8 and 5.5 kPa (Capstar-1000, CWE, Ardmore, PA). The left femoral artery was cannulated for measurement of arterial blood pressure and arterial blood gases (i-STAT; Hewlett Packard, Böblingen, Germany). The left femoral vein was cannulated and used for injections and infusions. The animals were killed by decapitation.

Experimental protocol

The experimental protocol is shown in Fig.1. The main study included 3 groups with 10 rats in each, where the plasma volume-expanding effect of 5% albumin was studied at 3 different arterial pressure levels during a state of increased permeability achieved by an intravenous injection of 0.5 ml dextran 70 (Macrodex 6%; Pharmalink AB, Upplands Väsby, Sweden) given after the surgical preparation [14]. As the decrease in plasma volume following a dextran injection reaches its maximum within 1 h [15], the albumin solution at a volume of 15 ml/kg was given 1 h after the dextran injection over 15 min. In group 1, no vasoactive drugs were given (control group). In group 2, noradrenalin infusion was started after the albumin infusion and continued throughout the experiment. Infusion of a mixture of the beta 1-blocking agent metoprolol (Seloken, Astra Zeneca, Mölndal, Sweden) and the alpha 2 agonist clonidine (Catapressan; Boehringer Ingelheim, Stockholm, Sweden) was started after albumin infusion in group 3 and continued throughout the experiment. The dextran injection resulted in a low arterial pressure (Fig. 2). Despite the volume substitution with albumin, the arterial pressure in the control group remained below baseline value throughout the experiment. Noradrenalin was given in a dose aimed at increasing in blood pressure to a value close to the value prevailing

before the dextran infusion. The dose of noradrenalin was in the range 0.5–2.2 μg/kg/min. After a bolus dose of 1 mg/kg and 1.0 μg/kg of metoprolol and clonidine, respectively, these drugs were infused at a rate of 1.0 mg/kg/h and 1.0 μg/kg/h. These doses are shown to have effective blood pressure reducing effects in the rat [16, 17]. The experiments were randomised but not blinded. In a separate group (n=9), an attempt was made to evaluate the effect of increased arterial pressure by noradrenalin infusion on plasma volume under conditions of hypovolemia and increased permeability. The experiments were similar to those in group 2 above, except that no albumin infusion was given. Central venous pressure was measured via the right internal jugular vein in 6 additional rats treated with dextran and albumin as described above, in an attempt to evaluate whether a change in hydrostatic pressure might have influenced the results through a change in venous pressure; 3 of the rats were given noradrenalin and 3 control rats were given normal saline.

Arterial blood gases were measured at baseline, 1 h after the dextran injection before the infusion of albumin, soon after the albumin infusion, and at the end of the experiment (Fig. 1). Plasma volume was measured 1 h after the dextran injection, before the infusion of albumin and at the end of the experiment.

The plasma volume (V) was calculated by measurement of the increase in radioactivity per ml of plasma (ΔC_2) after an intravenous injection of a known amount of activity of ^{125}I-albumin (C_1) [18]:

$$V = C_1/\Delta C_2$$

Radioactivity was measured with a gamma counter (Wizard 1480, LKB-Wallace, Turku, Finland). The increase in radioactivity was determined by subtracting the activity in a blood sample taken before the injection from that taken 5 min after the injection, taking into account the remaining radioactivity from previous plasma volume measurements. The blood was centrifuged and the radioactivity in a fixed volume of plasma was determined. To determine the exact dose injected, the remaining radioactivity in the emptied vial, the syringe and the needles was subtracted from the total radioactivity in the prepared dose.

Statistics

Results are presented as mean values ± SD. Statistical comparisons between groups were performed with one-way ANOVA, which was adjusted for multiple comparisons (Bonferroni). P-values less than 0.05 were considered significant. Sigma Stat 2.0 software was used.

Results

Physiological data

The data for haemoglobin (Hb), sodium (Na^+), potassium (K^+), pH, P_aO_2 and P_aCO_2 are summarized in Table 1. Hb increased from 129 ± 4 g/l before the dextran infusion to 146 ± 5 g/l one h after the dextran infusion ($p<0.05$) for the whole population in the 3 main groups (n = 30). After a decrease in Hb by the albumin infusion, the Hb value returned to baseline at the end of the experiment in the noradrenalin group, while it remained low in the control and in the metoprolol/clonidine groups. Na^+, K^+, pH, and P_aCO_2, were not significantly different between groups. P_aO_2 was lower in the noradrenalin group than in the other groups at the end of the experiment ($p < 0.05$). Mean arterial blood pressure at baseline, 1 h after the dextran injection, directly after the albumin infusion, 1 h after the albumin infusion and at the end of the experiment are presented in Fig. 2. Arterial pressure was higher in the noradrenalin than in the

control and metoprolol/clonidine groups ($p<0.01$). Blood pressures in the control and metoprolol/clonidine groups were lower than baseline values 1 h after the albumin infusion and at the end of the experiment ($p < 0.05$). The reduction in arterial pressure by metoprolol/clonidine relative to the control group was not statistically significant. In the noradrenalin group not given albumin, the noradrenalin infusion resulted in a blood pressure increase to values in average 7-10 mmHg lower than in the noradrenalin group given albumin (Fig. 2). In the experiments performed to measure central venous pressure, central venous pressure was 2.9 ± 0.5 mmHg at baseline, 2.7 ± 0.5 mmHg 1 hour after the dextran injection, 3.5 ± 0.4 mmHg 20 min after the albumin infusion and 3.6 ± 1.1 mmHg at the end of the experiment in experiments given noradrenalin. These values were not different from those in the control group, where central venous pressure was 2.8 ± 0.2 mmHg at baseline, 2.5 ± 0.8 mmHg 1 hour after the dextran injection, 3.4 ± 0.9 mmHg 20 min after the albumin infusion and 3.4 ± 0.8 mm Hg at the end of the experiment.

Plasma volume

Normal plasma volume in the male Spraque-Dawley adult rat, as measured in previous studies with the same technique as used in the present study, was 40–42 ml/kg [15, 19]. Plasma volume directly before infusion of albumin was 32.5 ± 3.5 ml/kg (n = 30), with no statistically difference between the 3 groups. The remaining increase in plasma volume 2.5 h after infusion of albumin at 15 ml/kg of albumin (PV_2-PV_1; Fig. 1) is shown in Fig. 3. It was 11.8 ± 3.6 ml/kg in the control group, 0.5 ± 6.3 ml/kg in the noradrenalin group and 12.5 ± 4.9 ml/kg in the metoprolol/clonidine group. The difference in plasma-expanding effect between the noradrenalin group and the other 2 groups was statistically significant ($p < 0.001$), but there was no difference between the metoprolol/clonidine group and the control group. In the experiments in which noradrenalin was given without plasma volume substitution, the plasma

volume decreased by 3.5 ± 3.0 ml/kg from the start of noradrenalin to the end of the experiment. Urine output after dextran injection was virtually zero in the control and the metoprolol/clonidine groups and <5 ml/kg in the noradrenalin group.

Discussion

The present study has shown that, during a state of increased permeability, increased arterial blood pressure by noradrenalin infusion aggravated loss of plasma fluid after treatment of hypovolemia with albumin. The blood pressure reducing effect compared to control of the metoprolol/clonidine group was insignificant, and there was no significant effect on plasma volume. The results are compatible with the observed changes in haemoglobon concentrations. Thus, in the noradrenalin group, the Hb concentration increased markedly during the experiment, indicating loss of plasma volume, while Hb remained unchange in the control and metoprolol/clonidine groups, indicating better-preserved plasma volume. Arterial oxygenation was impaired in the noradrenalin group compared to the other groups at the end of the experiment. The plasma volume-reducing effect of increased blood pressure by noradrenalin was less pronounced under hypovolemia when no albumin infusion was given.

The well-known permeability-increasing effect of dextran injection in the rat [14,15] was confirmed in the present study from the visually observed marked peripheral oedema that developed shortly after the dextran injection, by the large reduction in plasma volume, and by the reduction in blood pressure in combination with increased Hb.

The tracer albumin technique used is well established for measurement of plasma volume [18, 20]. The potential over-estimation of plasma volume because of transcapillary albumin escape

after the tracer injection must be small, due to the short 5-min period between injection of tracer and blood sampling, and should be equally large for all groups [15].

An increase in transcapillary leakage of plasma fluid under increased permeability after injection of dextran is compatible with the 2-pore theory of transcapillary fluid exchange [12]. As mentioned in the Introduction, this theory involves fluid and smaller solutes passing the capillary membrane through all pores, whereas proteins pass the membrane only through the much less common large pores. The transcapillary/transvenular hydrostatic pressure gradient is the dominant force responsible for fluid flow through the large pores, as the oncotic pressure gradient across these pores is very low. The proteins are lost to the interstitium mainly through convection, by following the large-pore fluid flux; there is always a continuous leakage of proteins, reflecting the normal transcapillary escape rate. Even a minute increase in total pore area by way of an increase in large pore area may cause a substantial increase in loss of proteins [12].

This hypothesis means that the leakage of proteins will increase with an increase in hydrostatic capillary pressure and the leakage will be greater at a high arterial or venous pressure than at low pressures. Transcapillary leakage of proteins could therefore be expected to be greater after an increase in arterial pressure, which may occur after volume expansion or during hypertensive treatment, and especially under increased permeability.

Our result with increased loss of plasma volume during noradrenalin infusion is compatible with the 2-pore theory. The infusion of noradrenalin resulted in an increase in blood pressure from subnormal values to values close to those at baseline (Fig. 2). The noradrenalin infusion resulted in loss of plasma volume that almost corresponded with volume of albumin infused,

resulting in a new hypovolemic state. This volume loss cannot be explained by an increased urine production, as virtually no urine production was observed. The fact that the loss of plasma volume was so large also means that even a smaller increase in arterial pressure could result in a significant loss of plasma volume. The fact that blood pressure was significantly lower than baseline values after the albumin infusion (Fig. 2) and plasma volume was relatively well preserved (Fig. 3) in the control group, may explain why metoprolol/clonidine treatment only showed a tendency to further reduction in arterial blood pressure and no further preservation in plasma volume.

As noradrenalin is a vasoconstrictor not only of the arterial resistance vessels, but also of the capacitance venous vessels, noradrenalin infusion may influence hydrostatic capillary pressure through alterations of venous pressure. Our results of just a small difference in central venous pressure between rats with or without noradrenalin indicate, however, that the venous effect on hydrostatic capillary pressure is small.

From the experiments showing a less pronounced loss of plasma volume by noradrenalin in uncorrected hypovolemia (the experiments with no albumin infusion), it seems, however, that the blood volume *per se*, directly or indirectly, influences the plasma volume losses associated with an increase in blood pressure. Thus, the loss of plasma volume when noradrenalin was given without prior albumin infusion was only 3-4 ml/kg, which can be compared with a loss of 14 ml/kg when albumin was given in a dose of 15 ml/kg. It must be noted, however, that the loss of plasma volume of 3-4 ml/kg during noradrenalin infusion was only slightly greater than the corresponding loss - which was virtually zero - when neither noradrenalin nor albumin was given, as shown in a previous study [15]. In other words, the higher plasma volume the larger will be the plasma volume loss at increased arterial pressure under increased permeability. We

can only speculate about possible underlying mechanisms. It can only marginally be an effect of difference in urine production as urine output was virtually zero in both groups. There may be a difference in increase in hydrostatic capillary pressure by noradrenalin between a normovolemic and a hypovolemic state. For example, the somewhat lower arterial pressure (Fig. 2) and a smaller venous pressure in the group not given albumin and a difference in post/precapillary resistance ratio may reduce the plasma volume loss. As a high haematocrit value may have a plasma volume-preserving effect [20, 21], the difference in haemoglobin concentration may also be involved.

The mechanisms underlying the worse arterial oxygenation in the noradrenalin group compared with the control and metoprolol/clonidine groups (Table 1) are clear. It may be an effect of increased lung water and/or uneven lung perfusion at a state of hypovolemia in combination with alpha-mediated vasoconstriction.

In addition to arterial and venous pressures, the hydrostatic capillary pressure is also a function of the post/precapillary resistance ratio [22]. It is well known that noradrenalin induces vasoconstriction not only in small arteries and arterioles but also in venules [22]. This means that the expected decrease in post/precapillary resistance ratio may be small in spite of the fact that there is a large increase in total vascular resistance; thus, the noradrenalin-induced increase in arterial pressure will result in a net increase in hydrostatic capillary pressure.

Because of reduced vascular tone, arterial pressure in patients with sepsis or SIRS often remains low despite adequate volume resuscitation. In these cases, noradrenalin is a common drug in intensive care, to correct arterial hypotension. According the present study, however, the noradrenalin infusion *per se* may increase the need for plasma volume substitution, leading

to a marked positive fluid balance, tissue oedema and worse clinical outcome [23-26]. Consequently, avoidance of unnecessarily high blood pressures preserves plasma volume, reduces the need for plasma volume expansion and may protect organ function. In fact, there is no evidence that higher values of blood pressure are of benefit in septic patients. This conclusion is supported by some recently published clinical studies, which have not shown improvement in renal function, splanchnic perfusion or oxygen variables when mean arterial pressure was increased with noradrenalin from 65 to 85 mmHg in volume-resuscitated patients [27, 28].

In conclusion, the present study using rats has shown that an increase in arterial pressure achieved by noradrenalin infusion increases the loss of plasma volume during a state of increased permeability. The plasma volume loss is smaller in uncorrected hypovolemia. Our results suggest that avoiding unnecessarily high blood pressures may save plasma volume and reduces the need for plasma expanders in patients with increased permeability. The results are compatible with the so-called 2-pore theory for transvascular fluid exchange.

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Table. 1. Haemoglobin (Hb), sodium (Na^+) and potassium (K^+) concentrations and also pH, P_aO_2 and P_aCO_2 at baseline, before albumin infusion 1 h after dextran injection, directly after albumin infusion, and 2.5 h later (at the end of the experiment).

		Hb g/l	[Na+] mmol/l	[K+] mmol/l	pH	P_aO2 kPa	P_aCO2 kPa
Control	Baseline	129.1±4.3	133.2±2.5	5.0±0.5	7.42±0.12	10.3±0.6	5.3±0.5
	Before albumin	146.3±5.4#	132.7±1.4	5.3±0.3	7.41±0.04	10.1±0.6	5.3±0.6
	After albumin	116.2±2.7	135.0±1.7	4.5±0.4	7.42±0.03	9.5±0.8	5.7±0.6
	End	115.0±8.4	135.6±1.3	4.9±0.3	7.40±0.11	9.7±0.8	4.9±0.5
NA	Baseline	129.8±4.9	134.6±1,9	4.7±0.4	7.43±.0.06	10.1±1.4	5.7±0.7
	Before albumin	144.3±9.3#	133.2±0.9	4.9±0.4	7.42±0.04	10.5±0.8	5.1±0.5
	After albumin	116.5±9.5	135.0±1.6	4.7±0.5	7.39±0.03	8.9±1.6	5.6±0.4
	End	132.5±5.7⊥	133.5±2.2	5.1±0.5	7.42±0.03	7.3±1.9***	5.2±0.4
M/C	Baseline	129.6±4.0	133.0±1.4	4.9±0.4	7.46±0.03	11.0±1.0	5.4±0.5
	Before albumin	145.6±9.3#	132.3±1.3	5.3±0.7	7.43±0.05	10.6±0.7	5.2±0.5
	After albumin	112.8±4.2	135.3±1.1	4.9±0.3	7.41±0.03	9.2±1.2	5.7±0.6
	End	115.0±7.0	134.9±1.5	5.1±0.7	7.44±0.03	10.1±0.7	5.1±0.2

NA: noradrenalin group; M/S: metoprolol/clonidine group.

difference compared to values at baseline, after albumin infusion, and at the end of the experiment [$P < 0.001$)

⊥ difference compared to corresponding value in the control and metoprolol/clonidine groups ($P < 0.01$).

*** difference compared to values at baseline, before and after albumin infusion, and at the end of the experiment in the noradrenalin group, and also corresponding values compared to control group and metoprolol/clonidine group ($P < 0.001$).

Legends

Fig. 1. Time scale of the experimental protocol. PV_1: plasma volume before albumin infusion. PV_2 – plasma volume at the end of the experiment. BGA: blood gas analysis.

Fig. 2. Mean arterial pressure for the control group, the noradrenalin group and the metoprolol/clonidin group. The symbol ⊗ shows mean arterial pressure at corresponding time points in the group given noradrenalin but not albumin.

difference compared to baseline and after albumin infusion in all groups ($p < 0.001$).

*** difference compared to the control and metoprolol/clonidine groups at the same time points ($P < 0.001$).

Fig. 3. Increase in plasma volume 2.5 h after the albumin infusion compared to the plasma volume just before the albumin infusion of 15 ml/kg for the 3 groups. It can be seen that the noradrenalin group had a much lower increase in plasma volume than the other 2 groups, which were not significantly different from each other. (n=11 in all groups). *** $p < 0.001$

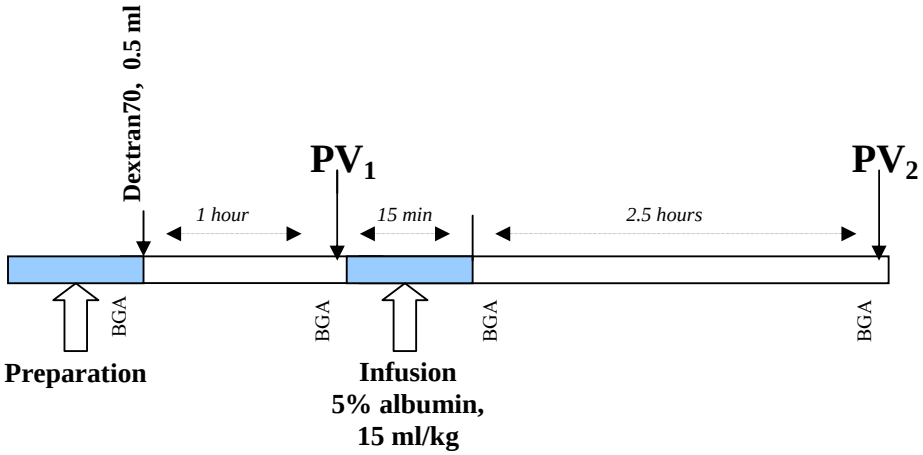
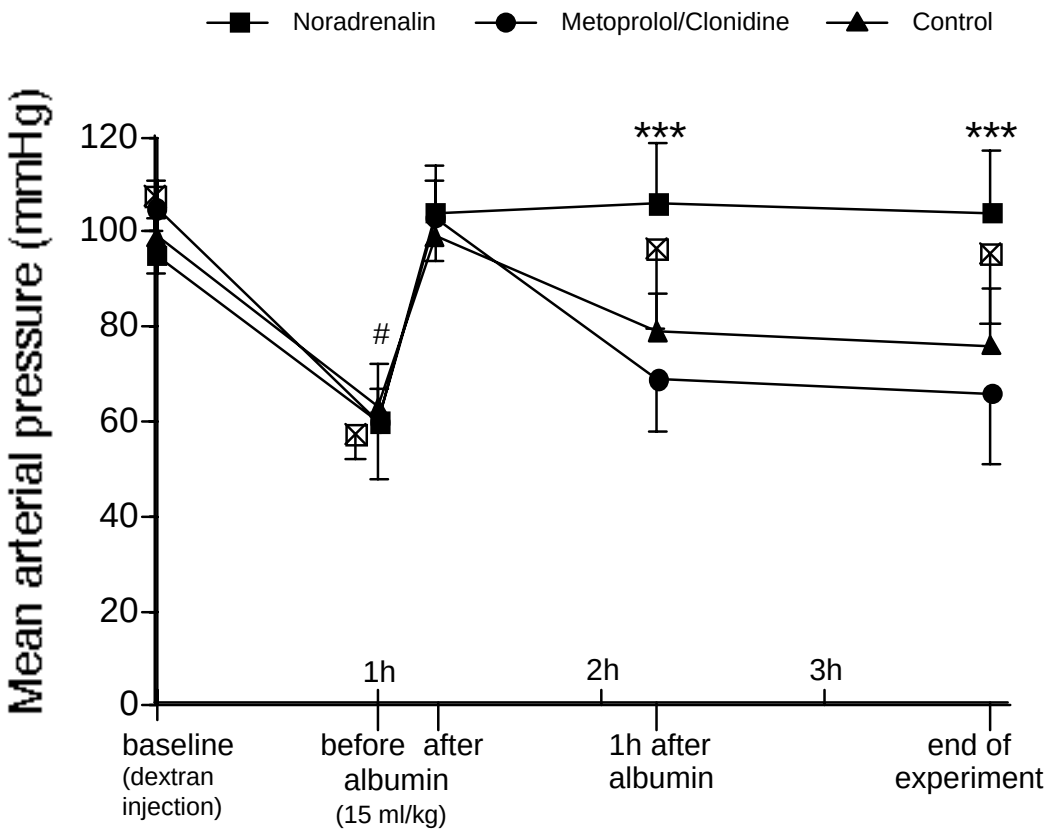

Dextran70, 0.5 ml
PV1
PV2
1 hour
15 min
2.5 hours
BGA
BGA
BGA
BGA
Preparation
Infusion
5% albumin,
15 ml/kg

Fig. 1.

Fig 2


Noradrenalin
Metoprolol/Clonidine
Control
Mean arterial pressure (mmHg)
120
100
80
60
40
20
0
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***
#
1h
2h
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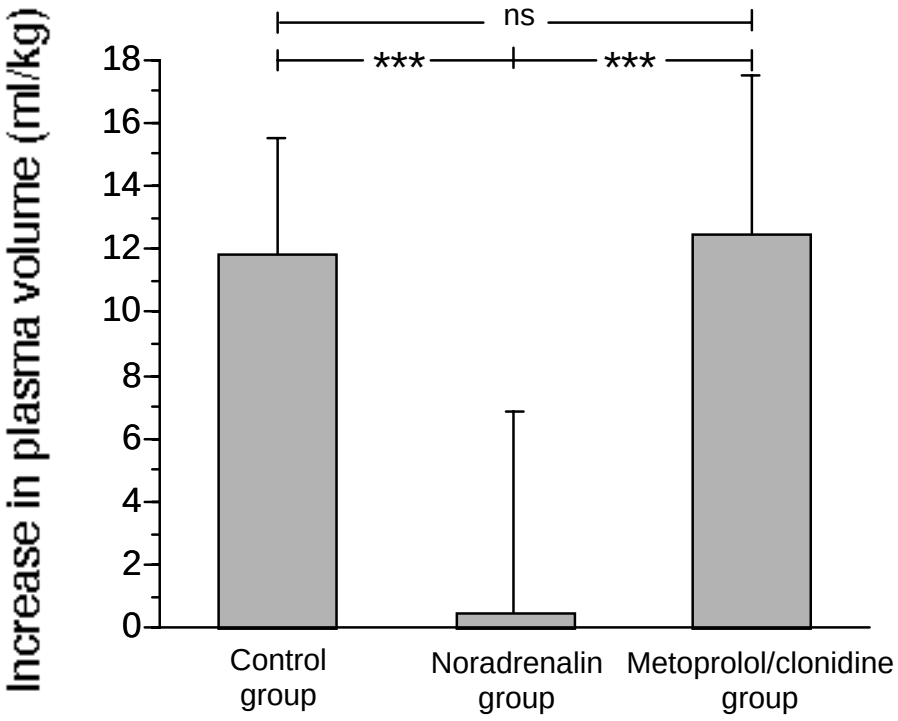
baseline
(dextran
injection)
before
albumin
(15 ml/kg)
after
1h after
albumin
end of
experiment


ns
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Increase in plasma volume (ml/kg)
18
16
14
12
10
8
6
4
2
0
Control group
Noradrenalin group
Metoprolol/clonidine group

Fig. 3