Chapter 2

Changes in vasomotor function of small coronary and mesenteric artery during 5-day follow-up after cardiopulmonary bypass in the rat. Characterization of endothelial mediators and contractility

Iryna V. Samarska, Hendrik Buikema, Hubert Mungroop, Martin C. Houwertjes, Fellery de Lange, Yumei Wang, Anne H. Epema, Robert H. Henning
Abstract

Background: Changes in vascular function may represent key elements of hemodynamic instability and organ injury after cardiopulmonary bypass (CPB). We investigated vascular contraction and endothelium dependent relaxation of small coronary and mesenteric arteries in a rat model of CPB with a follow-up period of 5 days.

Materials and Methods: Male Wistar rats (n=78) were anesthetized with isoflurane (2.5 %) and fentanyl/midazolam during CPB. Animals were assigned to sham or CPB with normothermic extracorporeal circulation for 60 min at a flow of 120 mL kg-1 min-1. After recovery for 60 min to 5 days, constriction to phenylephrine (mesenteric artery) and serotonin (coronary artery), and relaxation to acetylcholine (both arteries) were assessed. The expression of nitrotyrosine was assessed by western blot.

Results: Sham and CPB decreased the sensitivity to constricting agents at 1 day of recovery compared to control both in mesenteric (-log EC50; sham: 5.9±0.3, CPB: 5.9±0.1, control: 6.3±0.2) and in coronary arteries (-log EC50; sham: 5.8±0.3, CPB: 5.8±0.6, and control: 6.5±0.3). Moreover, CPB progressively decreased total ACh-mediated relaxation, the reduction becoming maximal at 2 days of recovery (AUC; sham: 248.8±48.8, CPB: 100.9±73.0), mainly due to decreased EDHF contribution. In addition, coronary artery of CPB animals displayed a biphasic change characterized by initial increased relaxation due to EDHF, followed by an inhibition of NO mediated relaxation. Nitrotyrosine content of mesenteric artery was about 8-fold higher at 2 days of recovery in CPB group compared to Sham.

Conclusions: In Sham animals, vasoconstrictor responses were primarily inhibited, demonstrating profound changes in vascular function following a relative mild procedure of anesthesia and cannulation. In addition, CPB inhibited endothelial-mediated vasorelaxation related to changes in EDHF and NO. Notably, vascular responses after CPB were not normalized at 5 days after the procedure. The observed changes may contribute to hemodynamic instability and organ injury during a protracted period after CPB.

Introduction

Cardiopulmonary bypass (CPB) enables complex cardiac surgical procedures. CPB however, is still associated with increased morbidity and mortality\(^1\) due to damage of various organ and systems including heart, kidney, lung, brain and gut.\(^2-9\) While the mechanisms initiating organ injury are complex and not fully understood, CPB induced activation of systemic inflammation and ischemia reperfusion injury are considered major factors. Previous research showed that this results in the impairment of vascular function immediately following CPB, consisting of both endothelial and smooth muscle dysfunction of small arteries.\(^10-19\) These changes affect organ blood supply contributing to the development of secondary tissue edema and tissue hypo-perfusion, setting the stage for multiple organ dysfunction. Thus, changes in vasoreactivity represent a pivotal component of the development of organ dysfunction following CPB.\(^20-22\)
Characteristic differences exist in the regulation of vascular tone in small artery beds of different organs. In general, vascular tone is controlled by neurohumoral factors acting through specific receptor pathways in the endothelium and smooth muscles, as well as by local hemodynamic factors including shear stress and intraluminal pressure.\textsuperscript{23,24} Further, vasodilation is governed mainly by the endothelium through the production of various components, including nitric oxide (NO), endothelium dependent hyperpolarizing factor (EDHF) and prostaglandins.\textsuperscript{25,26} The contribution of each component is dependent on the vascular bed studied, i.e. mesenteric artery is mainly dependent on EDHF, whereas coronary artery represents a mixed type dependent on NO and EDHF.\textsuperscript{27,28}

Clinical and experimental data suggest that the CPB induced initial systemic inflammatory response (SIRS) weans off within 24 h following surgery.\textsuperscript{29} In contrast, organ injury following CPB is observed not only in the immediate post-operative period (in the ICU),\textsuperscript{30} but during a protracted time frame following surgery.\textsuperscript{31, 32} Thus, we hypothesized that acute CPB injury results in protracted dysfunction of vasomotor control. Therefore, our objective was to evaluate vascular reactivity of different small arteries in an experimental rat model of CPB. Thus, we measured changes in vascular contractility as well as endothelial relaxation function in mesenteric and coronary arteries, including assessment of the contribution of different endothelial dilative components. Further, vascular reactivity was evaluated during a clinically relevant post-operative period. Therefore, time-dependent changes in vascular reactivity were examined from 60 min up to 5 days of recovery.

Material and Methods

The experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of Groningen. This study was performed in n=78 adult male Wistar rats with body weights of 507.4 ± 31.3 g (Harlan, Zeist, The Netherlands) housed under standard conditions with free access to food (standard rat chow; Hope Farms, Woerden, The Netherlands) and drinking water throughout the study period.

Experimental groups

Experimental animals were randomly allocated to one of four experimental groups with different duration of the recovery period after the procedure, being 60 min and 1, 2 and 5 days. Each group was subdivided in two sub-groups: CPB and Sham. CPB animals were subjected to the full experimental protocol described below, including anesthesia, cannulation, extracorporeal circulation, weaning and the corresponding recovery period. Sham animals followed the same procedure except for the extracorporeal circulation, but maintained cannulated and mechanically ventilated. In order to evaluate the normal vascular reactivity in rats without invasive interventions, an additional untreated control group was examined. Animals in this group were sacrificed under brief isoflurane anesthesia (2.5 %) only.
Experimental protocol

The experimental protocol consisted of the following parts: anesthesia, preparation, CPB, a weaning and a recovery period (see fig. 1). Anesthesia was induced with 2.5% isoflurane in O₂/air (1:1) before intubation and mechanical ventilation (Amsterdam Infant Ventilator; HoekLoos, Amsterdam, The Netherlands). Tidal volume was set to achieve normocapnia (verified by capnography 35-42 mmHg, and arterial blood gas analysis), with O₂/air (1:2) at a ventilation rate of 50 min⁻¹ (0.5 s inspiration time). Rectal temperature was kept at 37.0 ± 0.5 °C, using an electrical heating pad. The left femoral artery was cannulated (26-gauge catheter) for continuous blood pressure monitoring. The mean arterial pressure (MAP) was kept between 70 and 100 mm Hg by adjusting the isoflurane concentration as necessary (typically between 2.0-2.5%). Immediately before insertion of the arterial line, 250 IU kg⁻¹ heparin was administered. The left carotid artery was cannulated for arterial inflow using a 22-gauge catheter. A multi-orifice 4.5 French cannula (Desilets-Hoffmann catheter, Cook Son, The Netherlands) was advanced into the right heart using the right common jugular vein for access. The tail vein was cannulated for the administration of intravenous anesthetics, heparin, and protamine sulfate.

Cardiopulmonary bypass

Subsequently, CPB was initiated for 60 min. The set-up consisted of a glass venous reservoir, a peristaltic pump (Pericor® SF70, Verder, Haan, Germany), a rat membrane oxygenator (M.Humbs, Valley, Germany) and a glass counter-flow heat exchanger with built-in bubble trap. The oxygenator carried a sterile, disposable three-layer artificial diffusion membrane, made from hollow polypropylene fibres (Jostra AG, Hirrlingen, Germany). All components were connected with polyethylene tubing (1.6 mm inner diameter). The venous reservoir and heat exchanger were sterilized prior to use. The circuit was primed with 15 ml of haes 60 mg ml⁻¹ solution (Voluven®, Fresenius Kabi, Bad Homburg, Germany). No donor blood was used. Animals were additionally heparinized (250 IU kg⁻¹) after the start of ECC. During CPB, rats were anesthetized with intravenous fentanyl (10 μg kg⁻¹), atracurium (0.5 mg kg⁻¹), and
midazolam (2 mg kg\(^{-1}\)) and blood oxygen saturation was monitored continuously by a pulse oxymeter. Targeted CBP flow was 120 mL kg\(^{-1}\) min\(^{-1}\). During CPB ventilation was stopped and oxygenator gas flow was maintained at 800 ml min\(^{-1}\) of O\(_2\):air mixture (1:4). Samples for blood gas analysis (0.1 µl) were drawn at four time-points: at the end of preparation period (15 min before start of CPB), twice during the CPB (at 15 and 45 min), and at 45 min after the end of CPB.

**Weaning from CPB and recovery**

At the end, CPB flow was gradually decreased and mechanical ventilation was initiated. Protamine (150 IU kg\(^{-1}\) i.v.) was administered to neutralize heparin and cannules were removed and wounds sutured. Animals were kept ventilated under isoflurane anesthesia (0.8-1.0%) for 1 h to stabilize, followed by extubation. The total duration of the recovery period lasted 1 h, and 1, 2 and 5 days after the end of the CPB. Sacrification was performed under brief isoflurane anesthesia (2.5%).

**Vascular reactivity**

At sacrification, all mesenteric loops and the heart were removed and placed in cold physiological saline solution. Several segments of the third branch of the mesenteric superior artery and the interseptal coronary artery were dissected, prepared as ring vessel preparations (1.8-2.0 mm in length) and mounted on two 40 µm stainless wires connected to force transducers in individual organ bath chambers for isometric tension recordings in a wire-myograph (Danish Myo Technology A/S, Aarhus, Denmark). The baths contained 6 ml of Kreb's solution warmed to 37°C and bubbled continuously with 95%O\(_2\)/5%CO\(_2\) at pH 7.4. Vessels were subjected to a standardized normalization procedure \[33\] and left to equilibrate for 40 min until they were at a steady baseline. In brief, the vessels were distended stepwise until effective pressure exceeded 100 mmHg (13.3 kPa). The internal circumference, IC\(_{100}\), was found from the Laplace’s equation and the experiments were performed at the IC\(_1=0.9*IC_{100}\). Vascular segments were primed and checked for viability by two consecutive exposures to potassium chloride (60 mM).

The experimental protocol consisted of evaluation of contractile responses to phenylephrine (PE; 10 nM to 100 µM; mesenteric arteries) or serotonin (10 nM to 100 µM; coronary arteries). Endothelium-dependent relaxation to acetylcholine (ACh; 10 nM to 300 µM) was assessed in rings precontracted with PE (mesenteric artery) or serotonin (coronary artery). To assess the contribution of different endothelial mediators, ACh-induced relaxations were studied in the absence and presence of L-NMMA (0.1 mM; an inhibitor of NO-synthase) and/or indomethacine (1 µM; an inhibitor of cyclooxygenase) administered 20 min before application of phenylephrine. \[34\] It has been shown previously that the remaining ACh induced relaxation in presence of both cyclooxygenase and NO-synthase inhibitors was fully dependent on EDHF. \[35\] Following the final concentration of ACh, a maximal concentration of the NO-donor sodium nitroprusside (SNP; 0.1 mM) was applied to assess maximal endothelium-independent relaxation.
Western blot
The methods used were described previously. Briefly, after grinding, the frozen mesenteric beds were placed in 300 µl of boiling 2% SDS followed by pounding by a polytron (Kinematica AG Littau, Switzerland). Then, samples were centrifuged (4000 rpm, 1 min) and boiled (95°C) for 5 min. After a second centrifugation (13000 rpm, 3 min), supernatant was collected and used for measurements. Protein concentration was determined by Bio-Rad Dc Protein Assay (Bio-Rad, Hercules, CA). Twenty µg of total protein from each sample was separated on 10% Tris-Glycine-SDS-polyacrylamide gel and transferred to polyvinylidene difluoride (PVDF) membranes. Anti-nitrotyrosine antibody (ALEXIS Biochemicals, Lausen, Switzerland) and anti-GAPDH (Sigma, St. Louis, MO) were used as primary antibodies. Horseradish peroxidase-linked rabbit anti-mouse antibody (Sigma, St. Louis, MO) was applied as a secondary antibody. GAPDH served as a housekeeping protein.

Drugs
The composition of Krebs solution was as follows (mM): 120.4 NaCl, 5.9 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 25.0 NaHCO₃, 1.2 NaH₂PO₄, 11.5 glucose; these chemicals were obtained from Merck (Darmstadt, Germany). The following drugs were used: acetylcholine chloride (ACh), phenylephrine (PE), serotonin, indomethacine, SNP, N⁶-methyl-L-arginine (L-NMMA). Stock solution (10 mM) for indomethacine was prepared in 96% ethanol. All other drugs were dissolved in deionized water. L-NMMA was purchased from MP Biomedicals (Illkirch, France). All other compounds were purchased from Sigma (St. Louis, MO). The concentrations presented in the concentration-curve responses are expressed as a final molar concentration in the bath.

Data analysis
Data are given as mean ± SD. Contractile responses to PE and serotonin are presented in mN and relaxations to ACh and SNP were calculated as a percentage of pre-constriction. For each concentration-response curve the following parameters were determined: (1) the concentration at which half-maximal response was reached (EC₅₀), (2) the maximal response (Eₘₐₓ) and (3) the Area Under the Curve (AUC) in arbitrary units (AU) (SigmaPlot 11, Systat Software, San Jose, CA, USA). The difference in AUC of concentration-response curves to ACh in the absence and presence of inhibitor(s) was used to quantify the contribution of different endothelial relaxing components. Differences were evaluated using Student’s t-test, one-way ANOVA, repeated measurements ANOVA combined with post-hoc Bonferroni test or with t-test (SPSS 16.0, Chicago, IL, USA). Differences were considered significant at P<0.05 (2-tailed). The relationship between different relaxant pathways and total acetylcholine-mediated relaxation was evaluated with regression analysis and Spearman correlation.
Results

**Hemodynamics and blood gas analysis**

At baseline, all parameters measured were similar and in the normal range. During CPB, MAP was significantly lower compared with Sham (fig. 2). Blood gas analysis showed a decreased pH, bicarbonate (HCO$_3^-$), and Base excess and increased levels of pCO$_2$ and pO$_2$ during CPB (fig. 2), indicating mild acidosis. Hematocrit was significantly decreased in CPB groups as a consequence of hemodilution with the priming solution. Intraoperative hyperglycemia was observed in both Sham and CPB groups during the first 2 hours. After weaning from CPB, all parameters, including MAP, returned to normal values and were similar in both Sham and CPB, except for the hematocrit (fig. 2).

![Figure 2. Data on blood gas analysis and mean arterial pressure (MAP, femoral artery) and glucose concentration in Sham and CPB groups during the experimental protocol. Abbreviations: CPB = cardiopulmonary bypass. * indicates P<0.05, independent t-test.](image)
Contraction studies in isolated arteries

Full concentration–response curves to phenylephrine and serotonin were constructed in mesenteric (fig. 3, left panel) and coronary arteries (fig. 3, right panel), respectively. EC\textsubscript{50}-values and E\textsubscript{max} derived from these curves are presented in table 1.

As compared to mesenteric arteries of control rats, concentration–response curves to PE in Sham rats were shifted to the right (fig. 3A) and statistical significance was reached at 1h, 2 days and 5 days of recovery (table 1). In contrast, E\textsubscript{max} of PE was significantly decreased in Sham rats at 1 day of recovery, while normalizing again at longer periods of recovery (table 1). The time pattern of the changes in overall contractility to PE is shown in figure 3C as AUC for concentration–response curves to PE. In Sham rats, overall contractility was reduced most prominently at 1 day of recovery, followed by normalization at the 5\textsuperscript{th} post-procedure day. Contractile reactivity in CPB treated animals largely followed the same pattern (fig. 3B, table 1). Collectively, our data show that changes in contractility of mesenteric arteries were due to the anesthetic and cannulation procedure.

Concentration–response curves to serotonin in coronary artery of Sham rats (fig. 3D) were significantly shifted to the right after short-term recovery compared to control (i.e. at 1 h and 1 day; table 1). In contrast, E\textsubscript{max} for Serotonin in Sham was increased when studied after longer periods of recovery (i.e. at 2 days and 5 days), but not at short-term (table 1). Collectively, AUC of concentration-response curves show a tendency for a reduction in overall contractility in coronary arteries of Sham rats after acute recovery, followed by normalization towards the 5\textsuperscript{th} post-procedural day, but variation was too large to reach statistical significance (fig. 3F). Contractile reactivity in the group of CPB rats largely followed the same pattern (fig. 3E).

Table 1. Contractile response to phenylephrine in mesenteric arteries and to serotonin in coronary artery in Sham and CPB groups

<table>
<thead>
<tr>
<th>Recovery Time</th>
<th>-Log EC\textsubscript{50}</th>
<th>E\textsubscript{max}, µM</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Control</td>
<td>Sham</td>
</tr>
<tr>
<td>1 hour</td>
<td>-6.3±0.2</td>
<td>-6.1±0.5</td>
</tr>
<tr>
<td>1 day</td>
<td>-5.9±0.3*</td>
<td>-5.9±0.1*</td>
</tr>
<tr>
<td>2 days</td>
<td>-5.9±0.2*</td>
<td>-6.2±0.3</td>
</tr>
<tr>
<td>5 days</td>
<td>-6.2±0.3</td>
<td>-6.2±0.5</td>
</tr>
</tbody>
</table>

Data are given as mean±SD. Abbreviations: CPB, cardiopulmonary bypass; E\textsubscript{max}, the maximal response; -Log EC\textsubscript{50}, negative logarithm of the concentration at which half-maximal response was reached (EC\textsubscript{50}).*-P<0.05 vs Control, one-way ANOVA with post-hoc Bonferroni test # - P<0.05 vs Control, t-test
individual time points of Sham and CPB to controls showed a significant difference in EC$_{50}$ values at almost all time points in both groups (table 1). These data indicate that the protracted changes in contractility in coronary arteries were also due to the anesthetic and cannulation procedure rather than ECC. Collectively, the above findings demonstrate that CPB-related surgical procedures and/or extended anesthesia induced extensive and protracted changes in the contractility of mesenteric and coronary arteries, without significant additional influence of CPB.

*Relaxation studies in isolated arteries*

Endothelium-dependent relaxation was assessed by construction of full concentration-response curves to acetylcholine (ACh), shown in the figure in the Supplement Digital Content 1 for mesenteric and coronary arteries of normal control rats. The major endothelial dilative pathways were investigated by blocking the action of two components: vasoactive prostaglandins (PGs) and nitric oxide (NO) with indomethacin and additional pre-incubation with L-NMMA. The difference between AUCs of concentration-response curves in the absence and presence of inhibitors was used to calculate contribution of PGs, NO and endothelial derived hyperpolarizing factor (EDHF; Supplement Digital Content 1). Endothelium-independent relaxation was unaffected in both mesenteric and coronary artery since the relaxant response to sodium nitroprusside was similar in all groups (see table, Supplement Digital Content 2).

Preconstriction levels to phenylephrine were not significantly different among groups (see table, Supplement digital data 3, which is a table containing all preconstriction levels to phenylephrine). The changes in the AUC of total ACh-mediated relaxation and contributions of the endothelial dilative mediators in mesenteric artery were plotted against time of recovery (fig. 4). In Sham rats, total ACh-induced relaxation progressively increased during the recovery period to become even significantly larger as compared normal control rats (fig. 4A). In contrast, total ACh-mediated relaxation progressively decreased in CPB rats, the reduction becoming maximal at 2 days recovery (fig. 4A). Interestingly, the observed changes in total ACh-induced relaxation were similar to changes in EDHF contribution (fig. 4C), while contribution of NO and PGs did not change (fig. 4B, D). By the 5$^{th}$ day of recovery, AUC of total ACh-induced relaxation and the EDHF contribution had normalized again. Finally, EC$_{50}$ and E$_{max}$ of individual time points of Sham and CPB were compared to control by Student t-test (See table in Supplemental Digital Content 4), that showed significant changes in some point up to 5 days of recovery. The above findings indicate that the surgical procedure under general anesthesia induced a temporal increase in endothelium-dependent relaxation of mesenteric arteries via mobilization of EDHF, which is attenuated after CPB.
Preconstriction levels of coronary artery to serotonin were not significantly different among groups (See table in Supplement Digital Content 3). Total relaxation of ACh mediated dilatation was unaltered in Sham compared to control rats (fig. 5 A,C). Regarding its components, the NO-contribution to ACh-induced relaxation was profoundly decreased at 1 h in Sham rats and to a lesser extent in later time points (fig.
5D). However, decreased NO contribution was compensated by the increase in the contribution of dilatory PGs (fig. 5B).

Following CPB a more complex pattern was observed, resulting in an enhanced total relaxation to ACh at 1 h of recovery, followed by a larger inhibition of relaxation at 2 days (fig. 5A). Enhanced relaxation was mainly due to a significant increase in EDHF (fig. 5C), whereas inhibition at 2 days seems caused by a persistent decrease of NO contribution (fig. 5D).

Collectively, the above findings suggest that the unchanged relaxation observed following cannulation procedures and general anesthesia results from a balanced decrease in NO and increase in PGs. In addition, CPB mainly resulted in the acute mobilization of EDHF, thus enhancing relaxation at 90 min of recovery.

Western blotting

Reactive oxygen species (ROS) have been implicated to impair EDHF mediated relaxation of mesenteric artery. As CPB is associated with enhanced inflammatory response and oxidative stress, production of ROS was studied at 2 days of recovery, when relaxation in mesenteric artery was greatly enhanced in CPB compared to Sham (fig. 4). ROS production in mesenteric artery was quantified by measurement of nitrotyrosine content. Nitrotyrosine content was about 8-fold higher at 2 days of recovery in CPB group in comparison to Sham (fig. 6). Thus, these data suggest that CPB induced excess production of ROS at 2 days of recovery inhibiting the EDHF component of relaxation.

Figure 4. Total acetylcholine (ACh-) induced relaxation (A) - and the contribution of (B) prostaglandins (PG’s), (C) NO, and (D) EDHF hereto, all provided as AUC - in rat mesenteric arteries at different periods of recovery (1h, 1day, 2 or 5 days) following subjection of the animals to CPB-related procedures. Data are mean ± SD (n=5-8). * indicates P<0.05 CPB vs Control, t-test; # indicates P <0.05 Sham vs Control, one-way ANOVA with following Bonferroni test; † indicates P<0.05 CPB vs Sham, t-test. Abbreviations: CPB= cardiopulmonary bypass.
Figure 5. Total acetylcholine (ACh-) induced relaxation (A) - and the contribution of (B) prostaglandins (PG’s), (C) NO, and (D) EDHF hereto, all provided as AUC - in rat coronary arteries at different periods of recovery (1h, 1day, 2 or 5 days) following subjection of the animals to CPB-related procedures. Data are mean ± SD (n=5-8). * indicates P<0.05 Sham vs CPB, one-way ANOVA with following Bonferroni test; # indicates P<0.05 Sham vs Control, one-way ANOVA with following Bonferroni test; † indicates P<0.05 CPB vs Sham, one-way ANOVA with following Bonferroni test. Abbreviations: CPB=cardiopulmonary bypass.

Figure 6. Nitrotyrosine expression in mesenteric beds after 48h recovery period. representative blot (A) in Sham (n=3) and CPB (n=4) and Relative expressions patterns (B) in Sham and CPB samples. All data is normalized to expression level of GAPDH and present in arbitrary units. *P<0.05 CPB 2 days vs Sham 2 days, independent t-test.
Discussion

Changes in the vascular responsiveness of small arteries after cardiopulmonary bypass were studied during 5 days of recovery. First, our data show that CPB affects the vasomotor response of isolated arteries during a protracted time period, as vascular function following CPB did not normalize during 5 days of follow-up. Second, Sham and CPB induce complex changes in vasomotor response of small arteries following CPB in rat, which differ between vascular beds. Sham operation primarily induced changes in vasoconstrictor responses, with additional changes in vasorelaxation caused by CPB.

Sham operation caused a temporal depression in contractility of mesenteric and coronary artery around day 1 of recovery. In addition, sham operation caused an increased relaxant responsiveness of mesenteric artery after 2 days of recovery. In contrast, CPB strongly inhibited the vascular relaxation of mesenteric artery at 2 days recovery caused by decreased EDHF contribution.

Coronary artery of CPB animals displayed a biphasic change characterized by initial increased relaxation due to EDHF, followed by an inhibition of NO mediated relaxation. Collectively, these data demonstrate the induction of profound changes in vascular function following a relative mild procedure of anesthesia and cannulation. Specific changes induced by CPB consist of a change in endothelial-mediated relaxation, mainly related to its EDHF component. Furthermore, since the relaxant response to sodium nitroprusside was similar in all groups, alterations in relaxation seem due to changes at the level of the endothelial cell rather than caused by altered vascular smooth muscle responsiveness. Finally, the inhibited EDHF response in mesenteric artery of CPB animals was accompanied by the upregulation of nitrotyrosine, suggesting a role for an inflammatory response and ROS.

Vasoresponsiveness after CPB

Vascular reactivity after CPB has been investigated previously in various vascular beds although follow-up is limited to a few hours after CPB. In rat mesenteric artery, 90 min of CPB with 2.5 h recovery increased the contractile response to phenylephrine and evoked endothelial dysfunction. CPB at 28°C body temperature with aortic cross-clamping and cardioplegic arrest in dog caused depressed endothelial mediated mesenteric vasodilation immediately following CPB. The differences from our results, in which vasorelaxation was unaltered shortly following CPB, may be explained by differences from our model, i.e. a longer duration of CPB, cardioplegic arrest, hypothermia and the use of donor blood. Data on vascular reactivity of coronary artery following CPB are limited. In particular, a decreased myogenic vascular tone has been reported in pig coronary and human atrial arteries. However, data on changes in receptor mediated vasomotor responses following CPB are lacking, precluding comparison of our results to others.

Sham induced changes in vascular reactivity

One of the main findings of our study constitutes of changes in vascular reactivity following Sham procedure with a delay of 1 to 2 days following the procedure. Moreover, Sham induced changes appear more prominent in mesenteric artery. The impaired vascular function observed in isolated mesenteric artery favors vasodilation,
which in vivo may relate to a low flow state and splanchnicus hypoperfusion with increased incidence of gastrointestinal complications after major surgery.\textsuperscript{55}

The underlying mechanism of changes in vascular reactivity in Sham remains unknown. In our study, a main candidate for the induction of these changes is isoflurane, but limited data are available. Volatile anesthetics, including isoflurane, are well known for their vasorelaxing effect.\textsuperscript{56} Additionally, volatile anesthetics have been shown to inhibit AngII-mediated contraction.\textsuperscript{57} Studies employing administration of isoflurane to isolated arterial segments indicate that inhibition of contraction by isoflurane, if any, is limited to a short time interval of the recovery (up to 25 min).\textsuperscript{58,59} Given the time course of our experiments, including the 40 min equilibration time in the absence of isoflurane, it seems unlikely that recovery from prolonged isoflurane administration accounts for inhibited contractility of isolated arteries following the Sham procedure.

A second cause of the Sham-mediated changes in vascular reactivity might be an inflammatory response evoked by the procedure. Indeed, a Sham procedure of CPB in the rat results in a substantial increased plasma concentration of TNF-alpha up to 6 hours post-procedure,\textsuperscript{60} a cytokine inducing endothelial dysfunction through various mechanisms (reviewed in Zhang et al., 2009).\textsuperscript{61}

Collectively our data suggest that the relatively mild procedure of anesthesia and cannulation have a long-lasting impact on vasoresponsiveness of small arteries and warrant further studies to its mechanism and the contribution of specific anesthetics.

\textit{CPB induced changes in vascular reactivity}

The observation that CPB selectively affects endothelium dependent relaxation constitutes a second main finding of our study. Most likely, endothelial dysfunction is caused by the substantially aggravate systemic inflammation initiated by CPB,\textsuperscript{62-64} as increased plasma TNF-alpha concentrations relate to impaired endothelial mediated relaxation in rat.\textsuperscript{65} Further, impaired hemodynamics during CPB may add to vascular dysfunction, as demonstrated in a hypovolemic shock model in mice.\textsuperscript{66} Additionally, the observed changes in vascular reactivity might originate from metabolic disturbances during CPB. Slight changes in pH, HCO$_3^-$, and BE during CPB were observed and likely represent mild metabolic acidosis, which normalized after weaning from the CPB.

Our data imply that CPB affects the relaxation of mesenteric artery predominantly. This may be caused by the mesenteric artery being dependent on a single component of relaxation (EDHF), compared to coronary artery, which is balanced between NO, EDHF and prostaglandins. Thus, the compensatory capacity of the coronary vascular bed seems intrinsically superior over mesenteric artery. Possibly, the limited capacity of adjustment of mesenteric function relates to gastrointestinal complications after CPB, which is one of the serious complications with high mortality rate.\textsuperscript{67}

In summary, our study demonstrates that vascular responsiveness of small vessels was significantly altered during the entire follow-up period of 5 days following CPB in the rat. Not only CPB, but also Sham procedure causes protracted and
substantial alteration of vascular function. Changes in contractile and relaxation function of small arteries following CPB may lead to impaired control of vasomotor response, possibly related to hemodynamic instability and organ injury during the recovery period.

Reference List

Supplement Digital Content 1. Acetylcholine (ACh-)induced relaxation in the absence and presence of 10 μM indomethacin (INDO; i.e. to inhibit cycloxygenase-derived prostaglandins (PG’s)) only or with 100 μM L-NMMA (i.e. to inhibit NO production) additionally present, in arteries of normal control rats that underwent short anesthesia (for sacrifice) only. Full CR-curves are given for mesenteric (A) and coronary (B) arteries, as well as the individual contribution of different endothelial mediators hereto in both artery types (C), presented as the AUC (for further explanation and description, see text). Data are mean ± SD (n=5). Abbreviations: CPB, cardiopulmonary bypass.
### Supplement Digital Content 2

Table. Relaxant response to Sodium Nitroprusside of rat mesenteric and coronary arteries at different periods of recovery (1h, 1day, 2 and 5 days) in Sham and CPB groups

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<tr>
<th>Groups</th>
<th>Mesenteric artery</th>
<th>Coronary artery</th>
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<tbody>
<tr>
<td>Control</td>
<td>93.9±7.5</td>
<td>116.8±17.5</td>
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<tr>
<td>Sham 1h</td>
<td>95.2±12.6</td>
<td>99.1±16.5</td>
</tr>
<tr>
<td>CPB 1h</td>
<td>97.6±1.4</td>
<td>100.7±12.7</td>
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<td>Sham 1day</td>
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<tr>
<td>CPB 5days</td>
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<td>112.0±15.6</td>
</tr>
</tbody>
</table>

Data are given as mean±SD. Abbreviations: CPB=cardiopulmonary bypass.

### Supplement Digital Content 3

Table. Preconstriction level to phenylephrine of mesenteric and coronary arteries in different experimental groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Preconstriction level, mN</th>
<th>Mesenteric artery</th>
<th>Coronary artery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.9±3.5</td>
<td>2.3±0.93</td>
<td></td>
</tr>
<tr>
<td>Sham 1h</td>
<td>10.8±5.9</td>
<td>1.7±0.7</td>
<td></td>
</tr>
<tr>
<td>CPB 1h</td>
<td>9.7±3.7</td>
<td>1.4±0.9</td>
<td></td>
</tr>
<tr>
<td>Sham 1day</td>
<td>7.3±4.0</td>
<td>1.3±0.8</td>
<td></td>
</tr>
<tr>
<td>CPB 1day</td>
<td>6.6±3.1</td>
<td>1.3±0.9</td>
<td></td>
</tr>
<tr>
<td>Sham 2days</td>
<td>9.2±3.7</td>
<td>2.2±2.0</td>
<td></td>
</tr>
<tr>
<td>CPB 2days</td>
<td>10.5±4.9</td>
<td>1.5±0.9</td>
<td></td>
</tr>
<tr>
<td>Sham 5days</td>
<td>10.0±3.8</td>
<td>2.7±1.0</td>
<td></td>
</tr>
<tr>
<td>CPB 5days</td>
<td>8.8±5.7</td>
<td>2.7±1.9</td>
<td></td>
</tr>
</tbody>
</table>

Data are given as mean±SD. Abbreviations: CPB, cardiopulmonary bypass.

### Supplement Digital Content 4

Table. Characteristics of the ACh-mediated relaxation in coronary and mesenteric vessels during prolong recovery period. Comparisons were done with t-test vs Control group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Coronary artery</th>
<th>Mesenteric artery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E_{max}, % of the relaxation</td>
<td>-log EC_{50}</td>
</tr>
<tr>
<td>Control</td>
<td>54.8±16.6</td>
<td>-6.6±0.2</td>
</tr>
<tr>
<td>Sham 1h</td>
<td>58.0±8.3</td>
<td>-6.3±0.4</td>
</tr>
<tr>
<td>Sham 1d</td>
<td>51.5±22.8</td>
<td>-6.6±0.7</td>
</tr>
<tr>
<td>Sham 2d</td>
<td>53.8±21.6</td>
<td>-6.4±0.3</td>
</tr>
<tr>
<td>Sham 5d</td>
<td>39.9±9.3**</td>
<td>-6.0±0.2**</td>
</tr>
<tr>
<td>CPB 1h</td>
<td>72.5±11.0#</td>
<td>-6.6±0.3</td>
</tr>
<tr>
<td>CPB 1d</td>
<td>54.6±26.5</td>
<td>-6.3±0.7</td>
</tr>
<tr>
<td>CPB 2d</td>
<td>41.9±21.3</td>
<td>-5.9±0.4**</td>
</tr>
<tr>
<td>CPB 5d</td>
<td>53.3±21.5</td>
<td>-6.4±0.3</td>
</tr>
</tbody>
</table>

*P<0.05, t-test versus Control; **P<0.001, t-test versus Control; # P=0.051, t-test versus Control