Hypotension Caused by Extracorporeal Circulation  
Serotonin From Pump-Activated Platelets Triggers Nitric Oxide Release

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**Background**—Cardiopulmonary bypass and hemodialysis often cause hypotension. We investigated a possible role of pump-induced platelet activation with consequent serotonin release.

**Methods and Results**—In rats, a heparin-coated extracorporeal shunt was placed between the proximal part of a carotid artery and the distal part of a femoral artery. Autoperfusion did not affect platelets or hemodynamics. Pump perfusion, however, immediately elicited strong platelet aggregation, whereas aortic pressure rapidly fell to 60±12% (mean±SD) of its prepump value, partially recovered, and then progressively decreased to 70±12% at 2 hours. Femoral resistance doubled and then decreased to 59±11%. The initial changes in aortic pressure and femoral resistance were proportional to the amount of platelet aggregation, were accompanied by a rise (6-fold) in plasma serotonin levels downstream of the pump, but not in the aorta, and could be mimicked by serotonin-infusion into the leg. All hemodynamic changes were prevented or largely reduced by blockade of 5-hydroxytryptamine (5-HT)₂ receptors with pizotifen or ritanserin. The hypotension and femoral resistance decrease could also be prevented or abolished by inhibiting the production of nitric oxide (NO), an intermediate in 5-HT₂B receptor-induced vasodilation. When the extracorporeal blood was pumped into the aortic arch instead of the femoral artery, the hypotensive effect was similar and also NO dependent, but it was absent with venous return.

**Conclusions**—Pump perfusion with arterial return of the blood causes hypotension by endothelial NO-release, which in turn is triggered by serotonin from activated platelets. (Circulation. 2002;106:2588-2593.)

**Key Words:** platelets ■ endothelium ■ receptors ■ vasodilation ■ stress
Measurement of Platelet Behavior and Serotonin Levels

Platelet aggregation was continuously measured with a photometric device in the tube downstream from the pump, employing the increase in light transmission through flowing blood during passage of platelet aggregates. For quantification, the signals were corrected for changes in hematocrit (see below). After heparinization (Leo Pharma; 800 IU/kg), the proximal part of a carotid artery was cannulated and interconnected with medical grade polyvinyl chloride tubing (circum [ca] 45 cm long, 1.5 mm internal diameter [ID]) to the distal part of a femoral artery, creating blood flow by autoperfusion. Part of this shunt consisted of a peristaltic polyvinyl chloride tube (1.65 mm ID, Gilson), loosely positioned in a small noncommercial roller pump that was still switched off. The rotator of the pump had a diameter of 31 mm and contained 8 rollers with a diameter of 6 mm. All tubing was coated with Duraflo-II heparin by Edwards Lifesciences and was primed with gelofusine, a colloid osmotic solution (Braun). During the connection procedure, flow to the leg was interrupted for 2 to 3 minutes only. For transition from autoperfusion to pump flow, the peristaltic tube was compressed by tightenng the rollers of the pump with a calibrated screw until flow stopped; the pump was then started and the speed (ca 20 rpm) was adjusted to make flow equal to autoperfusion (ca 2 mL/min), as measured with an inline flow probe (1N, Transonic). Aortic and femoral pressure were measured with Statham P23 Db pressure transducers via stainless steel T-pieces in the shunt ~3 cm from the cannulas. Femoral resistance was continuously derived from the ratio of mean femoral artery pressure and flow, enabling the use of the leg for bioassay. Heart rate was derived from the aortic pressure pulse. Blood was shunted from a carotid artery into the inferior caval vein via a femoral vein in a second set of experiments, and from a femoral artery into the aortic arch via a carotid artery in a third set.

Statistics

Values in the text and legends are expressed as mean ± SD; the percentage values in figures and the table are expressed as mean ± SEM. Simple time series data were analyzed by 1-way ANOVA, whereas time series data with different conditions were analyzed with 2-way ANOVA, both for repeated measurements. For subsequent comparison of individual time-points, Bonferroni’s multiple comparison test was used. Differences were considered statistically significant if P < 0.05.

RESULTS

Hypotensive Effect of Pump Perfusion and Role of Platelet Aggregation

During autoperfusion into the femoral bed, no platelet aggregation was observed, and aortic pressure, heart rate, and femoral vascular resistance remained stable. On commencement of pump perfusion, however, platelets started to aggregate (Figure 1). Aggregation was maximal within the first 3 to 5 minutes, then leveled off but remained present for the next 2 hours of pumping. During the strong platelet aggregation, aortic pressure fell to about 60% of its initial value, and then partially recovered. Heart rate decreased temporarily to about 90% of initial (373 ± 23 b/min, P < 0.03). Femoral resistance showed a triphasic reaction; after a short decrease (<1
minute) and a 2-fold increase (about 5 minutes), it ended in a long-lasting decrease to about 60%. The rise in femoral resistance started 10 to 20 seconds before the fall in aortic pressure. Figure 2 presents mean changes during 2 hours of pump perfusion (solid squares, n=8) and open circles (ritanserin, n=5). Initial aortic pressure in mm Hg (mean±SD at time 0) was 135±10.5 (control), 96±16.5 (pizotifen), and 126±7.4 (ritanserin); initial femoral resistance in mm Hg · min · mL⁻¹ was 59±12.5 (control), 43±13.3 (pizotifen), and 53±30.1 (ritanserin). The differences between curves were highly significant (P<0.0001), both for the first 10 minutes and for 15 to 120 minutes.

During pump perfusion, platelet number and volume decreased by 18% (from 833±62 to 680±77 10⁹/L) and 4% (from 5.2±0.3 to 5.0±0.2 fl), respectively (P<0.0001, n=19). These changes did not occur during 2 hours of autoperfusion, ie, when the pump was off (n=6).

Figure 3 shows that both the initial pressure decrease and femoral resistance increase were linearly related to the amount of platelet aggregation, suggesting a causal relation.

Serotonin and 5-HT₃ Receptor Blockade

Figure 4A shows that shortly after pump start, the plasma serotonin level was strongly elevated in the femoral artery (from 12±6.5 nmol/L to 54±21 nmol/L in the first and 75±56 nmol/L in the third minute), but not in carotid blood (hatched bars). The elevation was positively related to the amount of platelet aggregation during the first 5 minutes (correlation coefficient [R]: 0.69, P<0.04, n=9). In the seventh and seventeenth minutes, when platelet aggregation had declined, serotonin levels in blood flowing into the hind leg had also declined (to 146±36% and 140±38% of prepump value, respectively; P<0.07), whereas systemic levels of its metabolite showed a significant incremental trend (Figure 4B).

Interestingly, the hemodynamic changes during pumping could be mimicked by serotonin infusion into the leg (Table). A dose of 4 to 6 µg · kg⁻¹ · min⁻¹ (right column) elicited a similar decrease in aortic pressure and rise in femoral resistance as observed shortly after onset of pump perfusion, and heart rate also slowed down to 90±2% of initial. A 5 to 10× lower dose (0.5 to 1 µg · kg⁻¹ · min⁻¹; left column), did not influence aortic pressure or heart rate but diminished femoral resistance to a similar extent as seen after some minutes of pump perfusion.

Blockade of 5-HT₃ receptors with pizotifen (Figure 2, open triangles, n=8) or ritanserin (open circles, n=5) inhibited or largely reduced the initial and later part of the pump-induced fall in blood pressure and the temporal decrease in heart rate, just as they blocked the effect of serotonin infusion into the leg (4 to 6 µg · kg⁻¹ · min⁻¹; Table). Because pizotifen lowered basal aortic pressure (to about 71% of initial), 3 additional experiments were performed, restoring pressure before pump start with vasopressin (ca 5 µg · kg⁻¹ · h⁻¹ IV). Also in these experiments, pizotifen prevented the pump-induced hypotension (pressure at 120 minutes: 104±5% of initial). Both antagonists also prevented...
the rise in femoral resistance and even turned it into vasodilation, but only partially reduced the long-lasting fall in femoral resistance (Figure 2B).

The 5-HT₂ receptor antagonists did not significantly diminish pump-induced platelet aggregation during the first 5 minutes (control: 1064±491, pizotifen: 1096±614, and ritanserin: 848±425 aggregates per mL blood). This indicates that their inhibition of the initial pressure and resistance changes (Figure 2) could not be ascribed to diminution of platelet aggregation, but were mediated by vascular receptor blockade.

**Nitric Oxide Synthase Inhibition**

Because vasodilation by 5-HT₂ receptor stimulation is known to be mediated by NO, we also inhibited NO-synthase (NOS). Figure 5 shows that NOS-inhibition completely prevented the decrease of aortic pressure and femoral resistance during pumping (P<0.0001, n=6), but did not abolish the initial vasoconstriction. The amount of platelet aggregates during the first 5 minutes (1069±510 aggregates per mL blood) did not significantly differ from control (1064±491). When L-NA was administered not before but at 2 hours after pump start (see arrow in Figure 5), pressure and resistance recovered and even showed an overshoot (from 70±11.7% to 117±10.7% and from 59±11.0% to 137±19.2%, respectively; n=6).

**Arterial Versus Venous Return**

Although the prevention of hypotension by 5-HT₂ receptor blockade indicates a role for serotonin, no significant increase in serotonin was observed in carotid artery blood during the first minutes after pump start (Figure 4). This suggests that factors other than serotonin are involved as well, eg, longer acting vasodilators released by local serotonergic stimulation of endothelium in the hind leg. When we bypassed the femoral bed by returning the extracorporeal blood directly into the inferior caval vein, the fall in blood pressure after pump start was indeed much smaller (see Figure 6, open
mediate in 5-HT2B receptor-induced vasodilation. 5,6. Our data pressure. Third, the hypotension could be prevented by 5-HT2 into the femoral artery could mimic the decrease in aortic infusion of blood into the femoral artery is shown (filled resistance importantly contributed to the pump-induced hypotension. First, the resistance after pump start (fall–rise–fall) corresponds well to the amount of platelet aggregation. Second, serotonin infusion into the femoral artery could mimic the decrease in aortic pressure. Third, the hypotension could be prevented by 5-HT3A receptor blockade or inhibition of NO production, the intermediate in 5-HT3B receptor-induced vasodilation. 5,6. Our data also indicate that during pumping NO-release was continuously elevated; when NO-synthase was inhibited after 2 hours of pumping, both aortic pressure and femoral resistance approximately doubled. These effects are too large to be solely caused by inhibition of normal NO production, which yielded increases of about 25% only.

Pumping elicited hypotension when the blood was returned arterially, either into the femoral bed or via the aortic arch, but had only little effect with blood returned into the caval vein (Figure 6). In the latter condition, an important part of the platelet-released serotonin was apparently cleared by the lungs. 20 With aortic return, the increased level of serotonin will have influenced resistance vessels in multiple organs. In case of femoral return, however, it is not immediately clear how serotonin caused systemic hypotension because it had to pass the lungs and its level had decreased to almost normal in carotid artery blood (Figure 4). Probably, the strong local serotonergic activation of endothelial cells in the leg elicited, via elevated nitric oxide production, release of longer-acting circulating factors, like NO-derived compounds 21 and/or prostacyclin. 22,23 Pilot experiments with femoral return indeed showed that the initial hypotension did not occur when 5-HT2 receptors in the femoral bed were locally blocked. Blockade was realized by infusing, during 5 minutes around pump start, with a low dose of ritanserin (5 μg/kg, n=2) or pizotifen (40 μg/kg, n=2) into the femoral return line. The efficacy of these doses was proven by the absence of changes in femoral resistance during an infusion of serotonin that still elicited systemic hypotension.

The 2 serotonin-2-receptor antagonists used in this study not only prevented the pump-induced hypotension, which is likely explained by their blockade of 5-HT3B receptors, but also the temporal rise in femoral resistance. This is probably the result of the close homology of the subclasses of 5-HT3 receptors, which causes antagonists like ritanserin and pizotifen to suppress the action of both the vasoconstricting 5-HT3A receptors and the vasodilating 5-HT3B receptors. 3,24–26 Because the latter are known to be more sensitive to serotonin than 5-HT3A receptors, the triphasic reaction of femoral resistance after pump start (fall–rise–fall) corresponds well to an increasing and decreasing serotonin concentration in the leg. The third phase of the reaction, ie, the long-lasting vasodilation, however, was only partially inhibited by 5-HT3 receptor blockade (Figure 2). This suggests that in the femoral bed, where serotonin concentrations would have been higher than in the systemic circulation, vasodilating serotonin receptors other than 5-HT3B were stimulated as well. Candidates are 5-HT1A receptors on the endothelium, 27 5-HT1B on sympathetic nerve endings, 24 and 5-HT7 on vascular smooth muscle cells. 5,28 When, however, in combination with 5-HT3 receptor blockade, all these receptors were inactivated simultaneously with, respectively, BMY 7378, 27 denervation of the leg, and lisuride. 28 Purperefuse still elicited some vasodilation, although serotonin-infusion did not (data not shown). Therefore, platelet release products other than serotonin, like ADP, were probably involved as well.

Platelet aggregation was strong during the first minutes after pump start, but then diminished. This diminishment was not caused by platelet consumption alone, as the loss of platelets after 2 hours of pumping was 18% only. The release of serotonin and the small, though highly significant decrease of mean platelet volume (from 5.2 to 5.0 fL), suggest that the ability of platelets to aggregate soon diminished as a result of
degranulation. Similarly, human platelets become less dense during cardiopulmonary bypass, apparently by release of serotonin and other granular contents; they seem to maintain their integrity, but lose functionality.

Clinical Interest
We showed that heparin coating of an extracorporeal device does not prevent the shear stress-dependent platelet aggregation and platelet loss induced by a roller pump. This may explain why bleeding problems in the clinical setting are not prevented by coating the system with heparin. The subsequent release of serotonin and the 5-HT\(_{2B}\) receptor-mediated NO-production might cause hypotension. This is especially true for cardiopulmonary bypass, where blood is returned into the aorta and clearance of serotonin is compromised by stagnation of pulmonary flow. However, patients on hemodialysis also seem at risk; although in our experiments on rats, hypotension remained absent when the blood was returned intravenously, as in hemodialysis, the clearance of serotonin by the lungs might be impaired in uremic patients because their basic level of serotonin is known to be elevated. It has indeed been reported that in cardiopulmonary bypass and hemodialysis, platelets are activated, and the levels of both serotonin and NO synthesis are increased. The prevention of pump-induced hypotension by 5-HT\(_{2B}\)-receptor blockade in this study suggests that 5-HT\(_{2}\)-receptor antagonists may be candidates for clinical use. The same might hold for inhibitors of NO-synthase, as suggested also by Peer et al, or substances able to prevent shear-induced platelet aggregation.

In conclusion, our animal studies with a heparinized extracorporeal shunt indicate that, at least in situations of arterial blood return, the hypotensive effect of pump perfusion is caused by endothelial release of NO, which in turn is triggered by serotonin from aggregating platelets.

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References
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