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

Piet Borgdorff, PhD; Durk Fekkes, PhD; Geert Jan Tangelder, MD, PhD

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

Use of extracorporeal systems in cardiopulmonary bypass and hemodialysis often causes severe hypotension. The etiology of this important predictor for mortality and morbidity is still unknown.12 One cause might be platelet aggregation with subsequent release of serotonin (5-hydroxytryptamine [5-HT]) and adenosine diphosphate (ADP). Although serotonin is often thought to induce vasoconstriction only,3 its vasodilating properties are important as well. The latter can be mediated by various receptors, of which the 5-HT2B receptors are the most sensitive and widespread. They stimulate endothelium-dependent release of nitric oxide (NO).4–6 In fact, several authors have reported platelet activation7,8 or increased levels of serotonin3,7 or NO9,10 during both intra-dialytic hypotension and cardiopulmonary bypass.

Platelets are activated even in heparin-coated systems,11,12 although such systems are known to reduce complement and contact activation,13,14 as well as platelet adhesion.15 Hence, factors other than blood-material contact must be involved. By monitoring platelet aggregation continuously, we were recently able to demonstrate that platelets in human and rat blood are activated by use of a roller pump.16 Furthermore, when a simple coated tube was placed between the proximal part of a carotid artery and the distal part of a femoral artery in rats, pump-induced platelet aggregation was accompanied by systemic hypotension, and both could be prevented with auranofin, a specific inhibitor of shear-stress–induced platelet activation that blocks the binding of von Willebrand factor to platelet glycoprotein Ib.17 In the present study, we measured the level of serotonin during pump-induced platelet aggregation within a heparin-coated shunt, tested whether the hypotension could be prevented by blockade of 5-HT2B receptors or inhibition of NO-synthase, and tested whether it could occur with venous or aortic return of the pumped blood.

Methods

Animals and Extracorporeal Circuit
Male Wistar rats (315 to 430 g; Harlan, Zeist, The Netherlands) were anesthetized with ketamine (Kombivet; 60 mg/kg IM) and pentobarbital (Nembutal; 35 mg/kg IP, followed by 10 to 14 mg/kg per hour IV). The animals were ventilated with an air/oxygen mixture (3:1) at 90 breaths/min. Tidal volume was adjusted to provide an arterial pCO2 of ∼40 mm Hg; arterial pH was between 7.35 to 7.39 and pO2 between 81 to 133 mm Hg. Body temperature was servo-controlled at 37.5°C. Handling of the animals was in compliance with the Guide
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.\(^1\) For quantification, the signals were converted to uniform spikes and counted over periods of 10 seconds. The raw signal, while Aggregates/sec presents the number of aggregates per second. Pump flow was set equal to the mean spontaneous flow.

For measurement of plasma serotonin, 0.4 mL blood was collected via both T-pieces, mixed with 0.1 mL K\(_3\)-EDTA (final concentration: 5 mmol/L), and centrifuged at 3300 \(g\) for 5 minutes at room temperature. The platelet poor plasma was decanted and frozen at \(-10^\circ C\) until analysis. The concentrations of serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were measured twice by high performance liquid chromatography (electrochemical detection with limit in plasma: 1 nmol/L). The mean recovery (\(\pm SD\)) of 5-HT added to plasma was 95\(\pm 7\)% and of 5-HIAA was 72\(\pm 8\)%\(^1\). The results were corrected for dilution using the change in hematocrit because each blood sample from the animal was replaced by an equivalent amount of gelofusine to avoid destabilization of blood pressure.

Serotonin-2-Receptor Blockade and NO-Synthase Inhibition

We used either pizotifen maleate (SanverTech; 3 mg/kg IP) or ritanserin (50 \(\mu g/kg\) IV) to block 5-HT\(_2B\) receptors. Dose-efficacy of the 5-HT\(_2\) blockers was tested in separate animals with 2 doses of serotonin (MSD), infused before the femoral cannula. NO-synthase was inhibited with Na\(-nitro-l\)-arginine (L-NA, 6 mg/kg IV). Ritan- serin, L-NA, and arginine vasopressin were obtained from Sigma-Aldrich. Ritanserin was dissolved in methanol (750 \(\mu g/mL\)) and subsequently diluted with saline to 75 \(\mu g/mL\). This vehicle had no effect on the hemodynamic variables or pump-induced responses. All other agents were dissolved in saline.

Statistics

Values in the text and legends are expressed as mean\(\pm SD\); the percentage values in figures and the table are expressed as mean\(\pm SEM\). 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\(\pm 23\) 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=6), showing that after half an hour, aortic pressure started to fall again, slowly approximating 70% of its prepump value; during this period, heart rate did not change. When, after 2 hours, the pump was switched off, aortic pressure and femoral resistance did not recover.

During pump perfusion, platelet number and volume decreased by 18% (from 833±62 to 680±77 10^9/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, i.e., 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-HT2 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-HT2B 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-HT\textsubscript{2} 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-HT\textsubscript{2} 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-HT\textsubscript{2B} 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

---

**Table 1. Effects of Serotonin Infusion**

<table>
<thead>
<tr>
<th></th>
<th>Time (min)</th>
<th>Aortic Pressure</th>
<th>Femoral Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.5–1</td>
<td>73±5.8%</td>
<td>306±12.5%</td>
</tr>
<tr>
<td>After pizotifen</td>
<td>0.5–1</td>
<td>no change</td>
<td>77±2.2%</td>
</tr>
<tr>
<td>After ritanserin</td>
<td>0.5–1</td>
<td>86±1.3%</td>
<td>70±2.2%</td>
</tr>
<tr>
<td></td>
<td>4–6</td>
<td>60±2.7%</td>
<td>85±1.3%</td>
</tr>
</tbody>
</table>

During autoperfusion, serotonin was infused for 2 minutes into the tube entering the femoral artery, both in control and after blockade of serotonin receptors (\(n=5\)). Resulting values of pressure and resistance are presented as mean±SEM percentage of pre-infusion value.
Role of Serotonin and Nitric Oxide in Pump-Induced Hypotension

Several of our results indicate that platelet serotonin release and increase of endothelial NO-production importantly contributed to the pump-induced hypotension. First, the degrees of initial hypotension and increase in plasma serotonin (4- to 6-fold) during the first minutes were both linearly related 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-HT$_2$ receptor blockade or inhibition of NO production, the intermediate in 5-HT$_{3A}$ receptor-induced vasodilation.$^5$ 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 long-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-HT$_2$ 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 $\mu$g/kg, n=2) or pizotifen (40 $\mu$g/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-HT$_{3A}$ receptors, but also the temporal rise in femoral resistance. This is probably the result of the close homology of the subclasses of 5-HT$_3$ receptors, which causes antagonists like ritanserin and pizotifen to suppress the action of both the vasoconstricting 5-HT$_{3A}$ receptors and the vasodilating 5-HT$_{3B}$ receptors.$^3,24,26$ Because the latter are known to be more sensitive to serotonin than 5-HT$_{3A}$ 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-HT$_2$–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-HT$_{3B}$ were stimulated as well. Candidates are 5-HT$_{1A}$ receptors on the endothelium,$^{27}$ 5-HT$_{1B}$ on sympathetic nerve endings,$^{24}$ and 5-HT$_{1C}$ on vascular smooth muscle cells.$^5,28$ When, however, in combination with 5-HT$_3$ receptor blockade, all these receptors were inactivated simultaneously with, respectively, BMY 7378, 27 denervation of the leg, and lisuride.$^{28}$ Pump perfusion 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

Discussion

Use of extracorporeal blood circuits in patients often causes hypotension, even when the system is coated with heparin. Simulating this serious clinical problem in a rat-model, we found that the platelet activation caused by a roller pump leads to serotonin release and, in case of arterial return of the blood, to a subsequent increase in nitric oxide production.
degranulation. Similarly, human platelets become less dense during cardiopulmonary bypass, apparently by release of serotonin and other granular contents;29 they seem to maintain their integrity, but lose functionality.8

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.11 The subsequent release of serotonin and the 5-HT2B 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.30 It has indeed been reported that in cardiopulmonary bypass and hemodialysis, platelets are activated,7,8 and the levels of both serotonin31,32 and NO synthesis are increased.9,10,31 The prevention of pump-induced hypotension by 5-HT2B receptor blockade in this study suggests that 5-HT2B-receptor antagonists may be candidates for clinical use. The same might hold for inhibitors of NO-synthase, as suggested also by Peer et al.,32 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.

Acknowledgments
We thank Edwards Lifesciences for coating our tubing with Dura-flow II heparin, and Astrid van Dalen for expert technical assistance.

References
11. Boonstra PW, Gu YJ, Akkerman C, et al. Heparin coating of an extracorporeal shunt indicates 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.
Hypotension Caused by Extracorporeal Circulation: Serotonin From Pump-Activated Platelets Triggers Nitric Oxide Release
Piet Borgdorff, Durk Fekkes and Geert Jan Tangelder

Circulation. 2002;106:2588-2593
doi: 10.1161/01.CIR.0000036082.04708.83

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/106/20/2588

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/