The Influence of Hemorrhage on Organ Perfusion during Deliberate Hypotension in Rats

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There is general concern that major blood loss during deliberate hypotension could produce severe organ ischemia, but documentation of the magnitude of this response remains obscure. To examine this response, we studied 43 male Sprague-Dawley rats that were divided into seven groups: the control animals received 1 MAC (1.4%) isoflurane only; the hypotensive animals received a 1.4% isoflurane baseline anesthetic and were then rendered hypotensive by either increasing the isoflurane concentration (dISO), or by adding sodium nitroprusside (SNP), or 2-chloroadenosine (2AD) to the baseline anesthetic, decreasing the MAP to 51 mmHg: hemorrhaged animals had hypotension produced in the same manner as for the hypotensive animals, but additionally were bled 20% of estimated blood volume during deliberate hypotension produced with either deep isoflurane (dISOH), sodium nitroprusside (SNPH), or 2-chloroadenosine (2ADH). After a 25-min period of hypotension, or hypotension plus hemorrhage, cardiac output and blood flow to brain, heart, gastrointestinal tract, kidney, and liver were measured with 14C-labelled 15-μm microspheres. Hypotension was associated with decreased blood flow to the kidneys in all groups and to the liver in the 2AD group and an increased blood flow to the heart in the SNP and 2AD groups. Hemorrhage decreased blood flow during deliberate hypotension to the brain and the gastrointestinal tract in the dISOH and 2ADH groups and to the liver in the dISOH group. Our results suggest that hemorrhage during deliberate hypotension with dISO or isoflurane plus 2AD may be associated with compromised organ blood flow, whereas blood flow to vital organs is maintained after 20% hemorrhage during isoflurane and superimposed SNP-induced hypotension. (Key words: Anesthetic techniques, deliberate hypotension: 2-chloroadenosine, hemorrhage, isoflurane, sodium nitroprusside. Blood pressure: hypotension. Brain: cerebral blood flow. Measurement techniques, regional blood flow: radioactive microspheres.)

DELIBERATE HYPOTENSION is used intraoperatively as an adjunct to anesthesia to decrease blood loss4–5 and to improve operating conditions.5 Deliberate hypotension also has been used extensively in the surgical management of intracranial aneurysms to reduce the risk of intraoperative rupture.6–8 Several drugs, including halothane,9 isoflurane,10,11 sodium nitroprusside,12,13 adenosine,14 nitroglycerine,15 trimethaphan,16 and labetalol,17 have been used to produce deliberate hypotension, although sodium nitroprusside and/or isoflurane appear to be the most commonly used agents.

Although there may be benefits to the reduction in arterial pressure, deliberate hypotension can alter organ perfusion.18,19 Intestinal infarction and hepatocellular damage have been reported as sequelae to profound deliberate hypotension in dogs.20 These concerns are magnified when there are other factors, such as concomitant hemorrhage, that could further reduce organ blood flow. For example, brisk hemorrhage from the intraoperative rupture of an intracranial aneurysm can complicate deliberate hypotension.21 In awake animals, acute blood loss causes increased sympathetic activity and a redistribution of cardiac output to the more vital organs.22 The presence of volatile anesthetics has been shown to affect this redistribution of cardiac output.23–26 Thus, major blood loss during deliberate hypotension could produce severe organ ischemia.

The effects of hemorrhage during deliberate hypotension have never been reported. This study was designed to determine whether there are differences in the effects of hemorrhage on organ blood flows during deliberate hypotension induced with sodium nitroprusside (SNP), deep isoflurane (dISO), or 2-chloroadenosine (2AD, a stable analog of adenosine). We determined these differences by measuring systemic and regional hemodynamic parameters during controlled hypotension and hypotension plus hemorrhage, in rats, using otherwise identical conditions.

Materials and Methods

Following institutional approval, 43 male Sprague-Dawley rats (353 ± 12 g) were divided into seven groups: those receiving 1 MAC (1.4%) isoflurane anesthesia only (control); those receiving dISO, SNP, or 2AD deliberate hypotension; and those that were hemorrhaged during hypotension with dISO (dISOH), SNP (SNPH), or 2AD (2ADH). All animals were anesthetized with isoflurane (1.4–2.0 vol% during surgical preparation; 1.4 vol% thereafter), and their lungs were ventilated with a rodent ventilator (Harvard Apparatus, Millis, MA; FLO2 = 0.3)
via a tracheostomy. Body temperature was maintained at 36–38° C by a heat lamp. Polyethylene cannulae (PE-50) were inserted into the left femoral artery and vein for measurement of arterial blood pressure and blood sampling and for the administration of fluids and drugs. The animals were paralyzed with pancuronium bromide (1 mg/kg iv). A Polyethylene catheter (PE-50), which had been tapered to a tip diameter approximately that of PE-10 tubings, was inserted into the left cardiac ventricle through the right carotid artery (the position was verified by pressure monitoring). Mean arterial pressure (MAP) and heart rate (HR) were recorded continuously using a pressure processor (Gould Statham Instruments, Hato Rey, Puerto Rico).

The inspired isoflurane concentration was maintained at 1.4 vol% for 30 min before the experiment was begun. The MAP in the treated rats was then decreased to 50 mmHg by infusing sodium nitroprusside or 2-chloroadenosine (10^{-5} or 10^{-3} M, respectively) in isotonic saline; the infusion rate was 1–2 ml/h. For animals receiving isoflurane induced hypotension, the inspired isoflurane concentration was briefly (2-3 min) increased to 5 vol% to reduce arterial pressure initially, and then maintained at a concentration (3.8 ± 0.3 vol%) that held arterial pressure constant at 50 mmHg. Saline (1.5 ml/h) was infused iv in the deep isoflurane and control animals so that fluid administration was identical in all groups. After 10 min of stable hypotension, 20% (1.4 ml·100 g body weight^{−1}) of the estimated blood volume was removed over 5 min in those hemorrhaged. The rate of administration of hypotensive agents was continued and held constant throughout hemorrhage and during a subsequent 10-min stabilization period.

The total duration of hypotension, or hypotension plus hemorrhage, was 25 min. Arterial PO_{2}, PCO_{2}, and pH were measured with a standard blood gas analyzer (Radiometer BMS Mark II, Radiometer American, Westlake, OH) in all animals after 15 and 30 min of stable anesthesia and at the end of the experiment. Hematocrit values were determined by the microhematocrit method. Cardiac output and organ blood flows were determined by the microsphere method\textsuperscript{27} after 25 min of hypotension in both the normovolemic and hemorrhaged rats. A well agitated suspension (0.5 ml) of approximately 400,000 11^{14}C-labelled microspheres (15 ± 1 μm; suspended in saline with 1% Tween 80) was injected into the left cardiac ventricle and the catheter flushed with 0.4 ml saline.

Cardiac output and organ blood flows were measured using the reference sample technique. Arterial blood was withdrawn by a constant withdrawal pump (withdrawal rate 0.01 ml/s) for 10 s before and 60 s after the injection of microspheres. The rats were then killed with iv KCl and the organs dissected free of fat and blood vessels and blotted on filter paper to remove excess blood. The position of the cardiac catheter was verified by direct observation. Similar blood flow rates in the left and right kidneys were used as a criterion for adequate mixing of microspheres; differences of more than 20% indicated insufficient mixing, and data from those animals were not included in the analysis. Measurement of radioactivity in the lungs was used to ascertain the absence of left-to-right shunting, perforation of the ventricular septum, or migration of microspheres through systemic capillary beds.

Radioactivity of reference samples and organs was measured in a well type gamma counter (Compugamma 1282-002, LKB Instruments, Gaithersburg, MD). The injected radioactivity was determined by subtracting residual activity in the empty catheter and syringe from the initial activity of the full syringe. Cardiac output (CO) was determined by the equation:

\[
CO = \frac{\text{total injected activity} \times \text{reference sample flow}}{\text{reference sample activity}}
\]

and is expressed in ml/min. Systemic vascular resistance was calculated as:

\[
\text{Resistance} = \frac{\text{MAP} - 10}{\text{organ blood flow}}
\]

and is expressed as mmHg · ml^{-1} · min. Organ blood flows (Q) were determined by the equation:

\[
Q = \frac{\text{organ activity} \times \text{reference sample flow}}{\text{reference sample activity}}
\]

and are expressed as absolute flow in ml·min^{-1}·100 g^{-1}. Vascular resistances of the organs draining into the central venous system were calculated as:

\[
\text{Resistance} = \frac{\text{mean arterial pressure}}{\text{cardiac output}}
\]

and are reported as mmHg · ml^{-1} · min · g. Central venous pressure was assumed to be zero, and thus perfusion pressure was assumed to equal MAP in these organs. Pressure in the portal system was assumed to be 10 mmHg following the work of Nakata, Leong, and Brauer,\textsuperscript{28} and therefore the formula:

\[
\text{Resistance} = \frac{\text{MAP}}{\text{organ blood flow}}
\]

was used to calculate resistance in the spleen, stomach, and intestines. The approach of Ross and Daggy\textsuperscript{29} was used for calculating liver blood flows. Radioactivity in the liver was assumed to represent hepatic arterial flow, whereas portal venous flow was calculated as the sum of blood flow through the spleen, stomach, and intestines. Total hepatic flow was calculated by adding hepatic arterial and portal venous flows.
ORGAN PERFUSION WITH HEMORRHAGE

TABLE 1. Arterial Blood Gas Values

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SNP</th>
<th>dISO</th>
<th>2AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>P_{a}O_{2} (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypotension</td>
<td>35 ± 1</td>
<td>34 ± 2</td>
<td>36 ± 1</td>
<td>35 ± 1</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>35 ± 1</td>
<td>34 ± 2</td>
<td>32 ± 1</td>
<td>34 ± 2</td>
</tr>
<tr>
<td>P_{a}CO_{2} (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypotension</td>
<td>91 ± 5^A</td>
<td>125 ± 7^C,^A</td>
<td>87 ± 5^A</td>
<td>95 ± 5^A</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>91 ± 5^A</td>
<td>111 ± 5</td>
<td>107 ± 5</td>
<td>118 ± 6^C</td>
</tr>
<tr>
<td>pH</td>
<td>7.37 ± 0.01^A</td>
<td>7.28 ± 0.04^C</td>
<td>7.34 ± 0.02</td>
<td>7.33 ± 0.02</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>7.37 ± 0.01^A</td>
<td>7.28 ± 0.03^C,^A</td>
<td>7.39 ± 0.05^A,^A</td>
<td>7.30 ± 0.01^I</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>37 ± 1</td>
<td>38 ± 1^I</td>
<td>35 ± 1^A</td>
<td>38 ± 1^I</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>37 ± 1^A,^LA</td>
<td>34 ± 1^C</td>
<td>34 ± 1^C</td>
<td>32 ± 1^C</td>
</tr>
</tbody>
</table>

Superscripts indicate significant differences (P < .05) compared with other groups: C = control; S = SNP; I = dISO; A = 2AD.

All data are reported as mean ± SEM. The results were analyzed using one-way analysis of variance to test for within group differences. If a significant difference was found, this was followed by Duncan’s multiple range test to compare specific groups. Significance was accepted for P < .05.

**Results**

The results for arterial blood gases and hematocrit are reported in table 1. P_{a}O_{2} was greater in animals receiving SNP than the other animals. The arterial pH was less than control in both the SNP and SNPH groups; the pH of the dISOH group was greater than the SNPH and 2ADH groups. The hematocrit of each hemorrhage group was less than control.

Cardiovascular data are reported in table 2. Hemorrhage during hypotension produced a MAP in the dISOH group (33 ± 1 mmHg) that was less than that in the SNPH (58 ± 5 mmHg) and 2ADH (47 ± 6 mmHg) groups. The heart rate in the 2AD and 2ADH groups was less than the other groups; the heart rate in the SNPH animals was greater than the others.

Cerebral blood flow changes are depicted in figure 1. Cerebral blood flow was not changed during hypotension with any of the agents. During hypotension and hemorrhage, cerebral blood flow remained unchanged in the SNPH group but decreased an average of 42 ± 7% from control in the dISOH and 2ADH groups.

Coronary blood flows are shown in figure 2. Hypotension was accompanied by an increase in coronary blood flow in the SNP and 2AD groups (average of both groups 135 ± 30%), but not in the dISOH group. Hemorrhage during hypotension accentuated the increase in the SNPH group, which increased coronary blood flow 308 ± 50% from control.

Renal blood flow data are reported in figure 3. Hypotension decreased renal blood flow (average over all groups 35 ± 7%) from control. Hemorrhage during hypotension decreased renal blood flow an average of 48 ± 6% from control in all groups.

Gastrointestinal (GI) tract blood flows are shown in fig-

TABLE 2. Cardiovascular Data

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SNP</th>
<th>dISO</th>
<th>2AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac output (ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypotension</td>
<td>104 ± 9</td>
<td>103 ± 8</td>
<td>88 ± 1^A</td>
<td>122 ± 6^A</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>104 ± 9^A</td>
<td>100 ± 7^A</td>
<td>62 ± 4^C,^A</td>
<td>75 ± 9^C,^A</td>
</tr>
<tr>
<td>Systemic vascular resistance (mmHg·ml⁻¹·min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypotension</td>
<td>0.78 ± 0.02^A,^LA</td>
<td>0.53 ± 0.03^C</td>
<td>0.60 ± 0.06^C,^A</td>
<td>0.42 ± 0.03^C,^I</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>0.78 ± 0.02^A,^LA</td>
<td>0.59 ± 0.06^C</td>
<td>0.54 ± 0.06^C</td>
<td>0.63 ± 0.07^C</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypotension</td>
<td>81 ± 7^A,^LA</td>
<td>53 ± 5^C</td>
<td>50 ± 1^C</td>
<td>50 ± 2^C</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>81 ± 7^A,^LA</td>
<td>58 ± 5^C</td>
<td>33 ± 1^C,^A</td>
<td>47 ± 6^A</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypotension</td>
<td>371 ± 7^A</td>
<td>387 ± 17^A</td>
<td>366 ± 5^A</td>
<td>248 ± 15^C,^A</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>371 ± 7^A</td>
<td>442 ± 24^A,^LA</td>
<td>357 ± 19^A</td>
<td>296 ± 16^A,^A</td>
</tr>
</tbody>
</table>

Superscripts indicate significant differences (P < .05) compared with other groups: C = control; S = SNP; I = dISO; A = 2AD.
Figure 4. Hypotension alone did not significantly alter GI tract blood flow in any group. However, hemorrhage during hypotension decreased GI tract blood flow an average of 39 ± 6% from control in the dISOH and 2ADH groups.

Total liver blood flows are depicted in figure 5. Liver blood flow decreased 32 ± 4% in the 2AD group, but was unchanged in the SNP and dISO groups. Hemorrhage decreased liver blood flow only in the dISOH group (44 ± 7%) as compared to control.

Organ vascular resistances are presented in table 3. Hypotension decreased vascular resistance to the brain in the SNP and dISO groups and to the heart in all groups. Hemorrhage during hypotension followed the same pattern but also increased kidney vascular resistance in the SNPH group.
study suggests that the choice of hypotensive technique may influence the extent of organ ischemia if hemorrhage occurs during deliberate hypotension.

In our study, 20% hemorrhage during deliberate hypotension decreased the MAP from the hypotensive level only in the dISOH group. This occurred because of an unchanged systemic vascular resistance (from hypotension) in the presence of a decreased cardiac output (compared with dISO). In the other groups, the MAP was maintained because of increased systemic vascular resistance during hemorrhage. In the 2ADH group the increase in systemic vascular resistance was enough to overcome a decrease in cardiac output. Presumably the stroke volume was decreased in all groups during hemorrhage, because dISOH and 2ADH were associated with a decreased cardiac output but unchanged heart rate (compared to hypotension), and the SNPH group with a stable cardiac output but increased heart rate (compared to hypotension).

Hemorrhage stimulates baroreceptor reflexes, increases sympathetic activity, increases circulating catecholamines, and activates the renin-angiotensin system. Actions of SNP on the baroreceptor reflex and the sympathoadrenal system may explain the response to hemorrhage during sodium nitroprusside hypotension. In the awake animal an immediate increase in heart rate is seen in response to sodium nitroprusside induced hypotension, but in our study and in other studies with a background general anesthetic, this did not occur. However, sodium nitroprusside induced hypotension enhances sympathetic activity and activates the renin-angiotensin axis. The priming of these systems by hypotension may exaggerate the response to superimposed hemorrhage, thus overcoming the inhibitory effects of anesthesia and producing the increased heart rate and maintaining the cardiac output.

Additionally, the sympathetic and baroreceptor effects of isoflurane and 2-chloroadenosine differ from those of

### Table 3. Organ Vascular Resistances (mmHg·ml⁻¹·min⁻¹·g⁻¹)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SNP</th>
<th>dISO</th>
<th>2AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>56 ± 6²</td>
<td>40 ± 3²</td>
<td>37 ± 3²</td>
<td>45 ± 5</td>
</tr>
<tr>
<td>Hypotension</td>
<td>56 ± 6²</td>
<td>34 ± 5²</td>
<td>42 ± 5²</td>
<td>54 ± 6²</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>29 ± 1²</td>
<td>9 ± 2²</td>
<td>14 ± 1²</td>
<td>9 ± 1²</td>
</tr>
<tr>
<td>Heart</td>
<td>29 ± 1²</td>
<td>6 ± 1²</td>
<td>10 ± 1²</td>
<td>11 ± 1²</td>
</tr>
<tr>
<td>Hypotension</td>
<td>20 ± 1</td>
<td>21 ± 2</td>
<td>20 ± 2</td>
<td>18 ± 1</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>35 ± 1</td>
<td>43 ± 7</td>
<td>43 ± 7</td>
<td>45 ± 7</td>
</tr>
</tbody>
</table>

Superscripts indicate significant differences (P < .05) compared with other groups: C = Control; S = SNP; I = dISO; A = 2AD.
sodium nitroprusside. Isoflurane suppresses the baroreceptor response in a dose-related manner, the high concentration required to induce hypotension inhibiting this response.\textsuperscript{35} In addition, lesser increases in circulating catecholamines and renin are seen during isoflurane hypotension compared with sodium nitroprusside hypotension, possibly contributing to the impaired reaction to hemorrhage seen with isoflurane.\textsuperscript{[1] With 2-chloroadenosine an increase in heart rate was inhibited, but an increase in systemic vascular resistance was not. The relative bradycardia may be explained by the apparent ability of 2-chloroadenosine to impair norepinephrine release from sympathetic nerves and to inhibit the sinoatrial node.\textsuperscript{36,37} The minimal increase in renin activity and lesser catecholamine levels found during hypotension with this drug may contribute to the responses to hemorrhage.\textsuperscript{34,37} 

Hemorrhage during hypotension decreased cerebral blood flow in the dISOH and 2ADH groups. Critical levels of cerebral blood flow have been defined for the onset of neurologic dysfunction,\textsuperscript{38} EEG silence,\textsuperscript{39} and cell membrane dysfunction,\textsuperscript{40} indicating the importance of absolute cerebral blood flow to avoid cerebral dysfunction.

Hemorrhage has been reported to decrease coronary blood flow and to increase coronary vascular resistance.\textsuperscript{41} No such change occurred in the presence of hypotension in our studies, suggesting a protective action with regard to myocardial perfusion during hypovolemia and deliberate hypotension.

Myocardial oxygen demand, as estimated by the product of heart rate and systolic blood pressure, was not significantly increased in any group. Since there was no decrease in global coronary blood flow in any experimental group, compared with control, it is likely that myocardial oxygen supply/demand balance remained adequate under all conditions. The question of coronary artery vasodilators inducing regional myocardial hypoperfusion in the presence of coronary artery stenosis was not addressed with this experimental model.

The kidney vasculature intrinsically autoregulates blood flow over a range of MAPs. There is also extrinsic control from the sympathetic nervous system, the renin-angiotensin system, and circulating catecholamines. Normal renal blood flow during 1 MAC isoflurane anesthesia has been reported in rat and swine,\textsuperscript{25,42} although a decrease has been reported in humans.\textsuperscript{43} We found a similar decrease in renal blood flow in all treatment groups. This probably reflects the decreased perfusion pressure and indicates that both the intrinsic and extrinsic renal regulation systems were unable to compensate for the actions of the hypotensive agents.

A previous study of the lower limits of deliberate hypotension resulted in intestinal infarction and hepatocellular damage in dogs.\textsuperscript{20} The results demonstrated that the abdominal vasa may be at significant risk for hypoperfusion during profound deliberate hypotension. In our study, the total GI tract blood flow remained intact with hypotension and decreased significantly during hemorrhage only in those receiving deep isoflurane.

Liver blood flow is a combination of hepatic arterial and portal venous flows. A decrease in portal blood flow is compensated by an increase in hepatic arterial flow, keeping total liver perfusion relatively constant.\textsuperscript{44} The inhibition of this compensatory mechanism has been reported for various anesthetics but not for moderate levels of isoflurane.\textsuperscript{45} In our study, only in the 2AD and dISOH groups did the total liver blood flow decrease and the compensatory mechanism appear to fail.

It must be emphasized that our experiments were performed in animals with a 1 MAC (1.4\%) isoflurane baseline anesthetic. Thus, the data we present for deliberate hypotension alone and hypotension complicated by hemorrhage must be interpreted in light of the fact that the hypotensive agents were superimposed upon animals already receiving isoflurane. The presence of isoflurane in these animals may have altered the response to hemorrhage, and thus, different results may be found with a different baseline anesthetic.

In conclusion, we found that when hemorrhage is superimposed upon deliberate hypotension induced with either deep isoflurane, sodium nitroprusside, or 2-chloroadenosine, deep isoflurane and 2-chloroadenosine are associated with decreased blood flow to the brain and GI tract, and additionally to the liver with deep isoflurane. In contrast, blood flows to brain, heart, kidney, GI tract, and liver are maintained after 20% hemorrhage during 1 MAC isoflurane anesthesia and superimposed SNP-induced hypotension. Although these data were obtained in rats only, the results imply that sodium nitroprusside may be preferable to either deep isoflurane or adenosine-induced hypotension if major blood loss should occur during deliberate hypotension.

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