Renal vascular response to haemorrhage in the rabbit after pentobarbitone, chloralose-urethane and ether anaesthesia

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Summary

1. Total renal blood flow and its cortical distribution were measured by the microsphere technique before and after haemorrhage in conscious rabbits, and after haemorrhage in rabbits anaesthetized with pentobarbitone, chloralose-urethane or ether.

2. The average blood loss necessary to achieve a fall in systolic blood pressure to about 65 mmHg was 101 ml in conscious rabbits and 38, 90 and 118 ml in weight-matched groups of rabbits anaesthetized with pentobarbitone, chloralose-urethane and ether respectively.

3. After haemorrhage in conscious rabbits total renal blood flow fell by 25%, this fall being confined to the superficial renal cortex.

4. In rabbits subject to haemorrhage under pentobarbitone anaesthesia renal blood flow fell by a further 23% when compared with the conscious bled rabbits. This reduction in blood flow was confined to the superficial cortex.

5. Haemorrhage in the rabbits subjected to chloralose-urethane anaesthesia caused no significant change in renal blood flow, as compared with conscious bled rabbits.

6. Haemorrhage under ether anaesthesia was associated with a further 33% fall in total renal blood flow, as compared with conscious bled rabbits. This was associated with a fall of 32% and 34% in superficial and deep cortical blood flow respectively.

7. Animals subjected to general anaesthesia may be particularly susceptible to the renal haemodynamic effects of haemorrhage.

Key words: anaesthesia, blood flow, chloralose-urethane, ether, kidney, microspheres, pentobarbitone.

Introduction

The mortality of acute renal failure in man remains high (Stott, Cameron, Ogg & Bewick, 1972; Kennedy, Burton, Luke, Briggs, Lindsay, Allison, Edward & Dargie, 1973) despite control of the biochemical manifestations of this disease by modern therapy. The search for preventive measures has led us to study some of the factors, such as haemorrhage, which may influence the development or severity of renal failure in animal models (Warren & Ledingham, 1975a).

The radioactive microsphere technique is a reproducible and sensitive technique for studying the renal circulation in the rabbit (Warren & Ledingham, 1975a) and can be used in conscious unrestrained animals standing quietly on a laboratory bench without the complications of bleeding, trauma, pain or changes in body temperature. The blood flow to the renal cortex is significantly reduced in these animals after anaesthesia with pentobarbitone, and both pentobarbitone and ether reduce the blood flow to the outer part of the renal cortex (Warren & Ledingham, 1975b). In contrast chloralose-urethane anaesthesia appeared to have no effect on renal blood flow or its distribution. We have
extended these observations to observe the effects of general anaesthesia on the renal haemodynamic response to haemorrhage in the rabbit.

**Methods**

Left atrial catheters (Warren & Ledingham, 1972) were inserted at least 2 weeks before the day of study in weight-matched groups of six to eight New Zealand White rabbits. These catheters were subsequently used for injection of room-temperature sodium chloride solution for cardiac output measurement by thermal dilution and for injection of radioactive microspheres. Aortic thermistors (Warren, 1974) were implanted into the lower aorta and sutured to the skin on the dorsum of the neck at least 3 days before an experiment. On the morning of an experiment a polyethylene catheter was sutured into a central ear artery under local anaesthesia for blood pressure and pulse-rate recordings and, in some animals, for measurement of blood gas partial pressures and pH.

All animals were allowed free access to food and water until the morning of the experiment. Five groups of rabbits were studied. The first group was conscious and intact, the second conscious and subjected to haemorrhage, and the last three groups were subjected to one of three general anaesthetics and also subsequently to haemorrhage.

Chloralose-urethane anaesthesia was induced by injecting a solution of urethane (2.81 mol/l) in sodium chloride solution (154 mmol/l; saline) slowly through the left atrial catheter at a dose of 5.61 mmol (500 mg)/kg body weight. This dose produced hypnosis but not surgical anaesthesia. The injection was followed by warm chloralose solution, 32 mmol (10 g)/l in saline at a dose of about 258 μmol (80 mg)/kg body weight. Sodium pentobarbitone anaesthesia was induced with a very slow left atrial injection of about 161 μmol (40 mg)/kg body weight, diluted in 12 ml of saline/kg body weight. In the doses used the anaesthesia was adequate to eliminate responsiveness to deep paw pressure. Ether anaesthesia was induced by allowing ether to drop on to a gauze-pad through which O\₂ + CO\₂ (95:5) was passed, and applying the mask containing the ether pad loosely to the face of the rabbit. Further details of the supervision of anaesthetized and conscious animals were given by Warren & Ledingham (1975b).

Initial haemodynamic variables were measured in all animals, before anaesthesia or haemorrhage, on two occasions at 15 min intervals. The mean of these two values appears as column A for each group of rabbits in Table 1. Repeat measurements were made once haemorrhage was complete in all animals, and after the same time in the control animals, and appear as column B in Table 1.

Pilot studies showed that if the rate of bleeding in conscious rabbits exceeded 2–3 ml/min, marked agitation occurred, sometimes followed by convulsions. Blood was therefore withdrawn at 2–3 ml/min from the ear artery catheter, with interruptions to record arterial pressure. Bleeding was discontinued once arterial pressure had fallen to 65–70 mmHg. The total volume of blood lost to achieve this blood pressure in the groups studied was a reflection of the sensitivity of that group to haemorrhage. Haemodynamic measurements, microsphere injections, blood gas determinations and renal blood flow measurements were all performed as described by Warren & Ledingham (1975a).

**Results**

There was no significant difference between body weight, cardiac output, mean arterial pressure or pulse rate in any of the five groups of rabbits studied before anaesthesia (Table 1). The cardiac output in these groups was within the normal range for conscious male New Zealand White rabbits (Warren & Ledingham, 1974). After a mean blood loss of 101 ml in conscious rabbits the output fell by 29% to 656 ml/min ($P < 0.01$), mean arterial pressure fell to 66 mmHg, a fall of 21% ($P < 0.01$), and pulse rate rose by 66 beats/min to 324 beats/min ($P < 0.01$). Blood gas partial pressures were normal in the conscious rabbits when the cardiac output was measured after haemorrhage (Table 1).

The cardiac output in the pentobarbitone-anaesthetized animals after loss of sufficient blood to reduce their mean arterial pressure to 69 mmHg was 657 ml/min, and this was no different from that in the conscious rabbits who were bled. The pulse rate did not change significantly in response to haemorrhage. The most striking difference was that the rabbits anaesthetized with pentobarbitone had lost only 38.2 ml of blood to achieve the same cardiac output as that in conscious animals who lost 101 ml of blood.

In the rabbits anaesthetized with chloralose-urethane the blood pressure, mean arterial pressure and pulse rate were not significantly different from the values in conscious rabbits who were bled. The mean loss was 90 ml/min to achieve a mean
Table 1. Body weight, haemodynamic data and arterial blood gas tensions in conscious intact rabbits, in conscious rabbits after haemorrhage and in groups subject to three different anaesthetics

Rabbits were bled until mean arterial pressure had fallen to just below 70 mmHg. A, Results in conscious animals before induction of anaesthesia. B, Results after completion of haemorrhage, or after an equivalent time in the intact group. Blood gas tensions were measured in at least four rabbits in each group, and from all rabbits anaesthetized with ether. Mean values ± SD are shown.

<table>
<thead>
<tr>
<th></th>
<th>Conscious intact (n = 6)</th>
<th>Conscious (n = 8)</th>
<th>Pentobarbitone (n = 8)</th>
<th>Chloralose-urethane (n = 6)</th>
<th>Ether (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>R</td>
<td>A</td>
<td>R</td>
<td>A</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>3787 ± 224</td>
<td>3843 ± 235</td>
<td>3889 ± 217</td>
<td>3846 ± 260</td>
<td>3607 ± 239</td>
</tr>
<tr>
<td>Cardiac output (ml/min)</td>
<td>888 ± 52</td>
<td>868 ± 51</td>
<td>874 ± 78</td>
<td>657 ± 77</td>
<td>923 ± 43</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>88 ± 4</td>
<td>86 ± 4</td>
<td>88 ± 3</td>
<td>69 ± 3</td>
<td>85 ± 4</td>
</tr>
<tr>
<td>Pulse rate/min</td>
<td>271 ± 27</td>
<td>289 ± 31</td>
<td>324 ± 27</td>
<td>271 ± 26</td>
<td>249 ± 24</td>
</tr>
<tr>
<td>PaO₂ (kPa)</td>
<td>—</td>
<td>—</td>
<td>15.5 ± 0.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PaCO₂ (kPa)</td>
<td>—</td>
<td>—</td>
<td>5.86 ± 0.4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>pH</td>
<td>—</td>
<td>—</td>
<td>7.43 ± 0.10</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Haemorrhage (ml)</td>
<td>—</td>
<td>—</td>
<td>101 ± 8</td>
<td>—</td>
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</tr>
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Renal effects of haemorrhage and anaesthesia
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**TABLE 2. Renal blood flow measurements in conscious intact rabbits, one conscious group and three anaesthetized groups subjected to haemorrhage**

The anaesthetics used were pentobarbitone, chloralose-urethane and ether. Total renal blood flow, flow to the superficial cortex (SC) and the deep cortex (DC) are shown separately. Blood flow is shown in absolute terms (ml/min) and also as the calculated flow (ml/min) per 100 g of tissue (ml min⁻¹ 100 g⁻¹). Results from right and left kidneys are combined. Mean values (+ SD in parentheses) are shown.

<table>
<thead>
<tr>
<th></th>
<th>Conscious intact group (n = 8)</th>
<th>Groups subject to haemorrhage</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Conconscious (n = 6)</td>
<td>Pentobarbitone (n = 6)</td>
</tr>
<tr>
<td>Total renal blood flow (ml/min)</td>
<td>113 (8-6)</td>
<td>85 (5-8)</td>
<td>65 (5-1)</td>
</tr>
<tr>
<td>Total SC flow (ml/min)</td>
<td>80 (4-8)</td>
<td>50 (3-4)</td>
<td>29 (4-2)</td>
</tr>
<tr>
<td>Total DC flow (ml/min)</td>
<td>33 (3-6)</td>
<td>35 (2-1)</td>
<td>36 (4-3)</td>
</tr>
<tr>
<td>Total SC flow</td>
<td>2-4 (0-5)</td>
<td>1-43 (0-2)</td>
<td>0-80 (0-1)</td>
</tr>
<tr>
<td>Total DC flow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SC flow (ml min⁻¹ 100 g⁻¹)</td>
<td>1231 (119)</td>
<td>669 (62)</td>
<td>310 (22)</td>
</tr>
<tr>
<td>DC flow (ml min⁻¹ 100 g⁻¹)</td>
<td>663 (52)</td>
<td>524 (43)</td>
<td>698 (29)</td>
</tr>
<tr>
<td>SC flow (ml min⁻¹ 100 g⁻¹)</td>
<td>1-85 (0-2)</td>
<td>1-27 (0-1)</td>
<td>0-44 (0-1)</td>
</tr>
<tr>
<td>DC flow (ml min⁻¹ 100 g⁻¹)</td>
<td></td>
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arterial pressure of 67 mmHg. This was not significantly different from the blood loss of 101 ml in the conscious group, to achieve the same mean arterial pressure.

In rabbits anaesthetized with ether, haemorrhage resulted in an insignificant rise in cardiac output, as compared with the control group subjected to haemorrhage, and mean arterial pressure and pulse rate were not significantly different. By contrast to the group anaesthetized with pentobarbitone, rabbits anaesthetized with ether tolerated a slightly larger haemorrhage than did the conscious rabbits. In all anaesthetized rabbits arterial blood $P_{O_2}$ and $P_{CO_2}$ were in the range 14·14-15·94 kPa and 4·92-5·91 kPa respectively (Table 1).

After haemorrhage total renal blood flow (Table 2) fell in the conscious group by 25% ($P < 0·01$) when compared with control rabbits. Flow to the superficial renal cortex (SC flow) fell by 37% ($P < 0·01$), but there was no significant change in flow to the deep cortex (DC flow), so that the ratio SC/DC flow fell by 41%. After pentobarbitone anaesthesia renal blood flow fell by 23% to 65 ml/min ($P < 0·01$) when compared with the conscious group after haemorrhage; total SC flow fell by 42% and DC flow remained unchanged when compared with the conscious bled group. The ratio SC/DC flow fell by 44% to 0·80. Pentobarbitone anaesthesia therefore potentiated the fall in SC flow in response to this fall in blood pressure, but did not change DC flow. In the chloralose-urethane group the response of the renal circulation to haemorrhage was not significantly different from that found in conscious bled rabbits. Ether anaesthesia was associated with a fall of 33% in total renal flow in response to a mean blood pressure of 68 mmHg. Total SC flow fell by 32% ($<0·01$) and total DC flow by 34% ($P < 0·02$), compared with the conscious group after haemorrhage. Thus ether anaesthesia potentiated the fall in both superficial and deep cortical flow after haemorrhage.

**Discussion**

*Effects of anaesthetics on the haemodynamic response to haemorrhage*

The most striking feature of the effect of pentobarbitone was that a total blood loss of 10 ml/kg body weight produced a fall in blood pressure and cardiac output which was almost identical with that obtained in the conscious rabbit after a loss of 26 ml/kg body weight. Although the pulse rate rose by
26% in conscious rabbits, there was no significant effect of haemorrhage on pulse rate in the rabbits anaesthetized with pentobarbitone. Several studies have shown impaired autonomic responsiveness under pentobarbitone anaesthesia, and suggest that reduced efferent vagal tone, blockade of sympathetic ganglia, reduced smooth muscle responsiveness, and possibly altered sensitivity of central vasomotor centres may all contribute to the haemodynamic effects of this anaesthetic (Cox, 1972a; Greisheimer, 1965; Korner, Uther & White, 1968). Our results suggest that pentobarbitone anaesthesia reduces reflex responsiveness to haemorrhage, although we cannot yet identify one mechanism responsible for this effect.

There was no difference between the fall in cardiac output in response to haemorrhage under chloralose-urethane anaesthesia when compared with that in conscious animals, and there was a similar rise in pulse rate. The volume of blood lost to achieve the same fall in arterial pressure was the same as in the conscious animals. Thus the autonomic response to haemorrhage of rabbits under chloralose-urethane anaesthesia is virtually identical with that found in normal conscious rabbits, confirming that autonomic reflexes are well preserved under chloralose-urethane anaesthesia (Korner et al., 1968; Cox, 1972b).

Our results suggest that rabbits anaesthetized with ether can tolerate a much larger blood loss (33 ml/kg body weight) than those anaesthetized with pentobarbitone (10 ml/kg body weight), chloralose-urethane (23 ml/kg body weight) or even conscious rabbits (26 ml/kg body weight) for a given fall in blood pressure. The fall in blood pressure and cardiac output was similar in both conscious and ether-anaesthetized rabbits, but the pulse rate was higher under ether anaesthesia, as noted by others (Liljestrand, 1953).

Effects of haemorrhage on the renal circulation

The fall in renal blood flow after haemorrhage was limited to the superficial cortex. Deep cortical flow remained unchanged, this component of the circulation exhibiting a remarkable capacity to autoregulate flow in spite of the fall in perfusion pressure to 64 mmHg. This pattern was noted qualitatively by Trueta, Barclay, Franklin, Daniel & Prichard (1947), although subsequent quantitative studies have usually shown some degree of deep cortical vasoconstriction after haemorrhage (Rector, Stein, Bay, Osgood & Ferris, 1972). Our studies suggest that the circulation to the deeper part of the cortex is well preserved after moderately severe haemorrhage, confirming that the resistance arterioles in the superficial and deep cortex are controlled by different mechanisms.

Both renal nerve stimulation (Pomeranz, Birtch & Barger, 1968) and experimental haemorrhage (Rector et al., 1972) can reduce the outer cortical blood flow although noradrenaline infusion (Stein, Boonjarern, Mauk & Ferris, 1973) reduce both superficial and deep cortical flow. \( \alpha \) and \( \beta \)-adrenoreceptor blockade do not consistently influence the renal haemodynamic response to haemorrhage, and it is not certain that the response to haemorrhage is due to increased noradrenaline secretion. However, as these previous studies were under pentobarbitone anaesthesia (Stein et al., 1973; Rector et al., 1972) they may well not describe the normal vasoconstrictor response to haemorrhage in conscious animals.

The renin content of glomeruli in the superficial renal cortex of the rabbit is much greater than in deep cortical glomeruli (Brown, Davies, Lever, Parker & Robertson, 1965). Renin secretion may rise in response to haemorrhage, as plasma renin activity is usually elevated (McKenzie, Lee & Cook, 1966). It is therefore possible that vasoconstriction in the superficial renal cortex is a consequence of renin secretion and possibly intrarenal conversion into angiotensin II.

Effects of anaesthesia on the renal haemodynamic response to haemorrhage

We have previously shown (Warren & Ledingham, 1975b) that pentobarbitone anaesthesia reduced total renal blood flow by 26 ± 4.6%, with a similar reduction in both superficial and deep cortical blood flow. Chloralose-urethane anaesthesia caused no change in total renal blood flow but some redistribution of flow from superficial to deep cortex. Blood flow to the deep cortex was well maintained in spite of pentobarbitone anaesthesia and haemorrhage in the present experiment, in contrast to the reduction in deep cortical blood flow observed with pentobarbitone anaesthesia alone (Warren & Ledingham, 1975b). These differences suggest that pentobarbitone, while diminishing total blood flow within the normal autoregulatory range of perfusion pressure, does not inhibit autoregulation of deep cortical flow under the stimulus of low perfusion pressure after haemorrhage. Thus resistance vessels in the deep cortex may have an intrinsic capacity for vasodilatation under conditions of low perfusion pressure. The failure of chloralose-urethane...
anaesthesia to affect the renal haemodynamic response to haemorrhage would suggest that this anaesthetic does not interfere with autonomic responsiveness, renin release or angiotensin sensitivity.

Ether anaesthesia potentiated the effects of haemorrhage on both superficial and deep cortical blood flow so that the ratio of superficial cortex/deep cortex flow did not differ significantly from that in conscious bled rabbits. In the unbled rabbit anaesthetized with ether the blood pressure was significantly higher than in conscious rabbits, as a result of sympatho-adrenal stimulation, and the decrease in superficial cortical flow probably represented potentiation of autoregulatory vasoconstriction in the face of this high blood pressure. Haemorrhage under ether anaesthesia caused overall renal vasoconstriction, in response to a mean arterial pressure of 64 mm Hg. Stimulation of renal nerves can cause overall cortical vasoconstriction in the dog (Stein et al., 1973), whereas haemorrhage redistributed flow from the superficial to the deep cortex. It therefore seems likely that the deep cortical vasoconstriction seen in these rabbit experiments is due to potentiation by ether of the normally insignificant influence of the renal nerves in the control of the renal haemodynamic response to haemorrhage, as suggested by the reduction in renal blood flow in dogs at low perfusion pressures under ether anaesthesia (Sasaki, Hashimoto & Iwatsuki, 1977).

Acknowledgments

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References


