Haemodynamic response to simulated haemorrhage in the rabbit: interaction of i.v. anaesthesia and hypoxia

D. W. Blake, A. F. Van Leeuwen, O. U. Petring, J. Ludbrook and S. Ventura

Summary

We have studied in eight rabbits the cardiovascular effects of midazolam, propofol and alfentanil with graded hypoxia. Central blood volume was reduced progressively by gradual inflation of a thoracic vena cava cuff so that cardiac index (Cl) decreased at a constant rate. Under control conditions the haemodynamic response was biphasic. During phase I, mean arterial pressure (MAP) was maintained by a progressive decrease in systemic vascular conductance (SVC). When Cl had declined to a critical level, phase II occurred with an abrupt increase in SVC and decrease in MAP. Phase I was prolonged by hypoxia, alfentanil and midazolam, but the effects were not additive. Phase I was shortened by propofol and this effect increased with hypoxia. The gradient of the SVC response in phase I was also reduced by propofol > midazolam, but not by alfentanil. The occurrence of phase II was less frequent during alfentanil infusion than midazolam and propofol with all of the inspired gas mixtures. Thus the opioid was protective against circulatory collapse with hypoxia and simulated hypovolaemia. (Br. J. Anaesth. 1995; 75: 610–615)

Key words


The net circulatory effects of acute hypoxia are a combination of direct local effects and central neural mechanisms [1, 2]. Reflex disturbances are caused by stimulation of the arterial chemoreceptors, increased central ventilatory drive, pulmonary afferent input, stimulation of the arterial chemoreceptors, increased mechanisms [1, 2]. Reflex disturbances are caused by combination of direct local effects and central neural

The net circulatory effects of acute hypoxia are a combination of direct local effects and central neural mechanisms [1, 2]. Reflex disturbances are caused by stimulation of the arterial chemoreceptors, increased central ventilatory drive, pulmonary afferent input, stimulation of the arterial chemoreceptors, increased mechanisms [1, 2]. Reflex disturbances are caused by combination of direct local effects and central neural

The net circulatory effects of acute hypoxia are a combination of direct local effects and central neural mechanisms [1, 2]. Reflex disturbances are caused by stimulation of the arterial chemoreceptors, increased central ventilatory drive, pulmonary afferent input, stimulation of the arterial chemoreceptors, increased mechanisms [1, 2]. Reflex disturbances are caused by combination of direct local effects and central neural

The net circulatory effects of acute hypoxia are a combination of direct local effects and central neural mechanisms [1, 2]. Reflex disturbances are caused by stimulation of the arterial chemoreceptors, increased central ventilatory drive, pulmonary afferent input, stimulation of the arterial chemoreceptors, increased mechanisms [1, 2]. Reflex disturbances are caused by combination of direct local effects and central neural
Hypoxia and hypovolaemia during anaesthesia

with halothane anaesthesia after induction with thiopentone 25 mg kg\(^{-1}\) and tracheal intubation. First, an inflatable cuff was placed around the inferior vena cava (caval cuff) via a right thoracotomy. Two to three weeks later a left thoracotomy was performed for placement of an ultrasonic (transit time) flow probe (type 6S, Transonic Systems Inc., Ithaca, NY, USA) around the ascending aorta. Experiments commenced at least 3 weeks after the second operation when the rabbits were well and gaining weight.

Insertion of catheters into an ear artery and ear vein, and retrieval of the tube to the caval cuff and the lead to the flow probe from their subcutaneous positions were performed under local anaesthesia (0.5% lignocaine). During saline and midazolam treatments, rabbits were restrained in a box placed in a 40-litre glass tank with inlet and outlet ports for the gas mixtures. In a previous study conscious rabbits did not become restless or agitated while breathing hypoxic gas mixtures [9]. Before alfentanil or propofol infusions, the trachea of the rabbit was intubated (4.0 mm Cole ET'T, Portex, Berch Sur Mer, France) after thiopentone 25 mg kg\(^{-1}\). This dose of thiopentone has only a short duration of action in the rabbit [10]. During alfentanil infusions the rabbits were also connected to a small animal ventilator (Phipps and Bird, Richmond, VA, USA), which was adjusted to maintain an end-tidal \(\text{P}_{\text{CO}_2}\) of 5.3 kPa. Anaesthetic or control infusions were continued for 60 min before the start of the studies.

**ANAESTHETIC INFUSIONS**

The four anaesthetic treatments were: propofol 8 mg kg\(^{-1}\) and 0.8 mg kg\(^{-1}\) min\(^{-1}\), alfentanil 0.04 mg kg\(^{-1}\) and 0.004 mg kg\(^{-1}\) min\(^{-1}\); midazolam 0.12 mg kg\(^{-1}\) and 0.012 mg kg\(^{-1}\) min\(^{-1}\) and 0.9% saline. The volume of each infusion was adjusted to 8–12 ml h\(^{-1}\). Methyl-scopolamine 0.05 mg kg\(^{-1}\) and 0.002 mg kg\(^{-1}\) min\(^{-1}\) was given with alfentanil to prevent the profound vagal bradycardia that otherwise occurred during hypoxia. Methyl-scopolamine effectively blocks peripheral muscarinic cholinoreceptors in the rabbit, but does not enter the central nervous system. In previous experiments this dose had no effect on each phase of the vasoconstrictor response to simulated haemorrhage [13].

The anaesthetic doses were determined by our previous experience of propofol and alfentanil in the rabbit and the dose of midazolam from its relative potency to produce anaesthesia in humans [14–16]. Depth of anaesthesia was tested by the righting reflex, eyelash and corneal reflexes and the response to noxious stimulus (tail clamp with forceps [10]).

**MEASUREMENT OF PHYSIOLOGICAL VARIABLES**

A Stratham P23Dc strain gauge placed at heart level was used to measure arterial pressure. The flow probe was connected to a small animal blood flow meter (model T206, Transonic Systems Inc., USA) to measure ascending aortic flow (cardiac output). Each probe had been calibrated in vitro with degassed saline. The accuracy of the electronic zero was tested immediately after the rabbits were killed and did not vary by more than 10 ml min\(^{-1}\) from true zero. Heart rate was measured by a tachometer actuated by the flow pulse. Signals were amplified and recorded on a Grass Model 7 Polygraph and sent to an Olivetti M24 computer with an A–D converter which provided 10-s mean values for arterial pressure (MAP), heart rate (HR) and cardiac output (CO). The computer also calculated 10-s means for cardiac index (CI = CO/body weight in kg) and systemic vascular conductance index (SVCI = CI/MAP × 100). \(P_{\text{aO}_2}\) and \(P_{\text{aCO}_2}\) were measured on 0.6-ml samples of arterial blood (Radiometer ABL3, Copenhagen, Denmark). Samples were obtained after 10 min exposure to each gas mixture, immediately before caval cuff inflation.

**ACUTE CENTRAL HYPOVOLEAMIA**

The caval cuff was inflated gradually by a micrometer-driven syringe so that CI declined linearly at a rate of approximately 8.5% of the baseline level per minute. This corresponds approximately to blood loss at a rate of 6 [11].

Caval cuff inflations were performed at 45-min intervals and commenced after 10 min exposure to the selected gas mixture. The inspired gas was room air during each 45-min rest period. The caval cuff was deflated when MAP had decreased to 40 mm Hg or after 8 min (at which time CI was about one-third of its baseline level). The SVCI response to cuff inflation was computed in preference to vascular resistance. A previous study found a linear relation between CI and SVCI, in contrast to vascular resistance, and that the vascular response to caval cuff inflation was similar to the response to acute blood loss in the rabbit [11].

**INSPIRED GAS MIXTURES**

Four mixtures of different concentrations of oxygen and carbon dioxide with nitrogen were tested daily: 21% oxygen (room air), 12% oxygen, 8% oxygen and 8% oxygen plus 8% carbon dioxide. Gas mixtures were delivered at 10 litre min\(^{-1}\) via the inlet port of the glass tank or to a reservoir bag connected to the ventilator inlet. Inspired oxygen and carbon dioxide were monitored by a Servo gas monitor 120 (Siemens–Elema AB, Solna, Sweden) and a Datex Normocap (Helsinki, Finland). Equilibrium in the inspired gas was reached in <5 min. Each rabbit was studied four times at intervals of at least 2 days. They were exposed to each of the four gas mixtures within each study and a different anaesthetic treatment was used in each study. The order of anaesthetic treatments and of the gas mixtures were randomized according to two 4 × 4 Latin squares.

At the end of the final study the rabbits were killed by an overdose of i.v. pentobarbitone sodium. At autopsy, in all rabbits the flow probe was found to be positioned correctly and the lungs were macroscopically normal.

**ANALYSIS OF RESULTS**

The following data were analysed for each anaesthetic treatment: (1) haemodynamic variables for
the 60 s immediately before introducing each gas mixture and, 10 min later, for the 60 s immediately preceding caval cuff inflation; (2) effect of the order of anaesthetic treatments and order of inspired gas mixture on haemodynamic variables before caval cuff inflation; (3) effect of anaesthetic on arterial blood-gas tensions and the change with each inspired gas mixture; (4) rates of change of CI, MAP, HR and blood-gas tensions and the change with each inspired gas mixture; (5) duration of phase I and level of SVCI at which phase II of the response to simulated haemorrhage commenced; (6) incidence of a clear phase II response.

Data were analysed by ANOVA with $P < 0.05$. Logistic regression was used to compare the incidence of phase II. In every case the between-rabbit effect was partitioned out in order to reveal the within-rabbit effects.

**Results**

The study with saline control and three anaesthetic infusions on separate days was completed in six of eight rabbits. The propofol infusion was omitted in one rabbit and the saline control in another because of injury and instrument failure, respectively. The Latin square design used to control for changes with time in resting circulatory variables over the four experiment days and during the 2–3-h anaesthetic exposures was effective as no systematic differences in the levels of MAP, CI or SVCI were found before commencing the different anaesthetic treatments or gas mixtures ($P > 0.4$).

Rabbits receiving propofol or alfentanil infusions did not respond to noxious stimuli and required tracheal intubation to maintain an unobstructed airway. Because of the respiratory depressant effect of alfentanil, positive pressure ventilation was also used during that infusion. During midazolam infusion, rabbits maintained an adequate airway without intubation and a response to noxious stimuli was still present despite the relative midazolam–propofol dose based on clinical experience.

During the midazolam, propofol and saline infusions the rabbits continued to breathe spontaneously and arterial blood-gas tensions remained within the normal range. With alfentanil infusion and controlled ventilation, $P_{aO_2}$ was lower than for the other treatment groups (table 1). The effects of the different inspired gas mixtures on arterial blood-gas tensions during the control saline infusion are shown in table 1. There was a progressive reduction in $P_{aO_2}$ and $P_{aCO_2}$ with 12 % and 8 % oxygen and the addition of carbon dioxide increased both $P_{aO_2}$ and $P_{aCO_2}$. Alfentanil infusion and controlled ventilation resulted in a lower $P_{aCO_2}$ at each inspired oxygen concentration compared with the other anaesthetic treatments and control ($P = 0.009$).

The effects of anaesthetic infusion on mean circulatory variables before exposure to the gas

<table>
<thead>
<tr>
<th>Infusion/gas</th>
<th>$P_{O_2}$</th>
<th>$P_{CO_2}$</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>12.4 (0.4)</td>
<td>4.4 (0.2)</td>
<td>7.4 (0.005)</td>
</tr>
<tr>
<td>Midazolam</td>
<td>13.6 (0.7)</td>
<td>4.3 (0.2)</td>
<td>7.47 (0.02)</td>
</tr>
<tr>
<td>Propofol</td>
<td>11.1 (0.7)</td>
<td>4.9 (0.2)</td>
<td>7.39 (0.02)</td>
</tr>
<tr>
<td>Alfentanil</td>
<td>8.5 (1.0)</td>
<td>5.5 (0.9)</td>
<td>7.41 (0.06)</td>
</tr>
<tr>
<td>21 % $O_2$</td>
<td>12.4 (0.4)</td>
<td>4.4 (0.2)</td>
<td>7.4 (0.01)</td>
</tr>
<tr>
<td>12 % $O_2$</td>
<td>6.8 (0.3)</td>
<td>3.5 (0.1)</td>
<td>7.50 (0.02)</td>
</tr>
<tr>
<td>8 % $O_2$</td>
<td>4.7 (0.3)</td>
<td>2.5 (0.1)</td>
<td>7.60 (0.02)</td>
</tr>
<tr>
<td>8 % $O_2$ + 8 % $CO_2$</td>
<td>5.6 (0.2)</td>
<td>6.7 (0.3)</td>
<td>7.26 (0.01)</td>
</tr>
</tbody>
</table>

$P$ values for the effect of the interaction of anaesthetic and inspired gas on CI and SVCI were 0.5 and 0.002, respectively. Inspired gas mixtures maintained for 10 minutes were: 21 % oxygen ( ), 12 % oxygen ( ), 8 % oxygen ( ), 8 % oxygen and 8 % carbon dioxide ( ).

<table>
<thead>
<tr>
<th>Infusion/gas</th>
<th>MAP (mm Hg)</th>
<th>HR (beat min$^{-1}$)</th>
<th>CI (ml min$^{-1}$ kg$^{-1}$)</th>
<th>SVCI ($100$ mm Hg l min$^{-1}$ kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>80 (1)</td>
<td>225 (3)</td>
<td>165 (4)</td>
<td>208 (6)</td>
</tr>
<tr>
<td>Midazolam</td>
<td>81 (1)</td>
<td>252 (4)</td>
<td>162 (7)</td>
<td>200 (8)</td>
</tr>
<tr>
<td>Propofol</td>
<td>60 (3)</td>
<td>221 (5)</td>
<td>169 (7)</td>
<td>294 (12)</td>
</tr>
<tr>
<td>Alfentanil</td>
<td>84 (2)</td>
<td>242 (8)</td>
<td>150 (8)</td>
<td>178 (9)</td>
</tr>
<tr>
<td>21 % $O_2$</td>
<td>82 (2.1)</td>
<td>226 (8)</td>
<td>171 (11)</td>
<td>209 (15)</td>
</tr>
<tr>
<td>12 % $O_2$</td>
<td>84 (2.5)</td>
<td>231 (7)</td>
<td>169 (9)</td>
<td>200 (14)</td>
</tr>
<tr>
<td>8 % $O_2$</td>
<td>84 (4.4)</td>
<td>180 (10)</td>
<td>157 (11)</td>
<td>191 (19)</td>
</tr>
</tbody>
</table>

$P$ values were between infusions and between gases (ANOVA)
mixtures are shown in table 2. During saline and midazolam infusions the haemodynamic variables were within the normal range for our laboratory. However, infusion of propofol decreased MAP by 27 % with an increase in SVCI (56 %). During alfentanil infusion, CI and SVCI were reduced by 9 % and 14 %, respectively.

Ten minutes exposure to hypoxic gas mixtures in unanaesthetized rabbits produced only a small (12 %) increase in MAP with combined hypoxia and hypercapnia and an increase in heart rate with hypoxia (table 2). However, hypoxia altered the effects of the different anaesthetics on MAP (P = 0.003) and SVCI (P = 0.002). The increase in MAP with hypoxic mixtures was present during midazolam and alfentanil infusion, but was reversed with propofol. CI was not influenced by the inspired gas during saline or anaesthetic infusions, but there was a further increase in SVCI with hypoxia and alfentanil infusion. Heart rate was maintained in the alfentanil group with i.v. hyoscine preventing vagal responses during hypoxia.

The gas mixtures did not alter CI significantly, although CI remained lower than for conscious controls after alfentanil (fig. 1).

**SIMULATED HAEMORRHAGE**

MAP, HR, CI and SVCI responses to caval cuff inflation in a typical rabbit breathing 21 % oxygen are illustrated in figure 2. The SVCI responses during saline, propofol and midazolam infusions can be divided into a phase of progressive vasoconstriction (phase I), followed by a sudden decompensation and increased conductance (phase II). Phase II did not occur with infusion of alfentanil.

The type of anaesthesia influenced the duration of phase I (P < 0.001), the slope of the SVCI response (P < 0.001) and the level of SVCI at the end of phase I (P = 0.01), (fig 3, table 3). The duration of phase I with the anaesthetic treatments was in the order alfentanil > midazolam > saline > propofol (table 3). The slope of the systemic vascular conductance index response was alfentanil = saline treatment > midazolam > propofol. SVCI at the end of phase I was greater with propofol infusion than with the other treatments.

Hypoxia prolonged the duration of the phase I vasoconstrictor response (fig 3, table 3; P < 0.001). However, there was an interaction between the effects of anaesthesia and hypoxia (P = 0.001) so that hypoxia had no further effect during propofol or alfentanil infusion. Hypoxia did not alter significantly the slope of the SVCI response.

The incidence of a clear phase II decompensation was less during alfentanil infusion than with saline, midazolam or propofol for all of the inspired gas mixtures (P = 0.026, logistic regression).

**Discussion**

In the rabbit, the relatively minor haemodynamic response to severe hypoxia and absence of the compensatory phase II [9] suggest that centrally mediated responses override local vasodilatation [17]. The primary aim of this study was to test how these central responses might be altered by different types of anaesthesia and by combined anaesthesia and hypovolaemia. Although there were marked differences in the haemodynamic responses to the three types of anaesthetic studied, the pattern was not changed by hypoxia. However, the differences were exaggerated during combined hypoxia and hypovolaemia so that the effects of centrally mediated reflex responses were reduced to a greater extent by propofol than midazolam, and were potentiated by alfentanil.

The four inspired gas mixtures produced moderate and severe hypoxia and hypoxia with hypercapnia. The lower arterial oxygen tension with each gas mixture during alfentanil infusion was probably related to the use of mechanical ventilation and increased ventilation–perfusion inequality. However, the more severe hypoxia in this group would have biased the results in the opposite direction to the main findings of the study. Combined hypoxia and alfentanil infusion resulted in episodes of profound bradycardia in rabbits with
parasympathetic function intact. We decided to block vagal responses with methyl-scopolamine during alfentanil infusion to maintain heart rate. The increase in heart rate with hypoxia and hypovolaemia was then similar in each of the treatment groups and the cardiac output and vasoconstrictor responses could be compared. Reflex bradycardia during chemoreceptor stimulation may dominate other cardiovascular effects.

The propofol infusion maintained anaesthesia in the rabbits with absence of response to noxious stimuli, but without significant depression of ventilation despite a dose approximately four times that required for surgical anaesthesia in humans. The midazolam dose was chosen to be equivalent according to the relative potencies of the drugs in humans, but still did not induce surgical anaesthesia in the rabbit. The alfentanil dose depressed respiration and blocked responses to noxious stimulation. Although the anaesthetic effects differed, the relative circulatory effects of the anaesthetic infusions were similar to those observed in humans. It is therefore reasonable to infer that the pattern of their effects on circulatory reflex responses may also be similar.

The major effects of the anaesthetics on the resting circulation were an increase in SVCI associated with decreased MAP during propofol anaesthesia, and reduced CI and SVCI with alfentanil. Hypoxia alone increased MAP, probably because of a reflex sympathetic effect. The main interaction between the effects of anaesthesia and hypoxia was the further increase in SVCI with propofol and hypoxia compared with similar small reductions in SVCI with midazolam and alfentanil infusions or hypoxia and saline infusion.

In conscious rabbits two phases were identified in the vascular conductance response to simulated haemorrhage. There was a progressive decrease in conductance with maintenance of MAP to a critical level of CI, when vasoconstriction failed and MAP decreased precipitously. The initial decrease in

<table>
<thead>
<tr>
<th>Infusion/gas</th>
<th>Time</th>
<th>SVCI</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>219 (20)</td>
<td>159 (10)</td>
<td>-14.0 (1.2)</td>
</tr>
<tr>
<td>Midazolam</td>
<td>324 (37)</td>
<td>137 (23)</td>
<td>-10.0 (0.6)</td>
</tr>
<tr>
<td>Propofol</td>
<td>191 (23)</td>
<td>274 (50)</td>
<td>-5.2 (4.2)</td>
</tr>
<tr>
<td>Alfentanil</td>
<td>418 (28)</td>
<td>77 (9)</td>
<td>-13.0 (2.4)</td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.014</td>
</tr>
<tr>
<td>21 % O₂</td>
<td>219 (20)</td>
<td>159 (10)</td>
<td>-14.0 (1.2)</td>
</tr>
<tr>
<td>12 % O₂</td>
<td>256 (22)</td>
<td>152 (13)</td>
<td>-10.9 (1.2)</td>
</tr>
<tr>
<td>8 % O₂</td>
<td>380 (27)</td>
<td>100 (7)</td>
<td>-15.1 (3.6)</td>
</tr>
<tr>
<td>8 % O₂ + 8 % CO₂</td>
<td>447 (11)</td>
<td>88 (8)</td>
<td>-13.8 (1.2)</td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.001</td>
<td>0.7</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Table 3 Phase I of systemic vascular conductance index (SVCI, ml min⁻¹ kg⁻¹ mm Hg⁻¹) response to simulated haemorrhage for saline and anaesthetic infusions in rabbits breathing air and saline infusion and hypoxic gas mixtures (mean (SEM)). Time = time in seconds until loss of vasoconstriction, MAP < 40 mm Hg or cuff released at 7 min; SVCI = minimum conductance reached; Slope = regression of SVCI on time.
SVCI depends on arterial baroreceptor function [4], and this was modified to varying degrees by the anaesthetic drugs tested. Alfentanil prolonged phase I, so that a greater reduction in conductance was achieved and the onset of a phase II decompensation was less likely. With a normal inspired oxygen concentration, alfentanil therefore assisted arterial pressure maintenance during haemorrhage. The response during midazolam infusion was intermediate between the alfentanil and control (saline) responses. Because of the hypotension and increased vascular conductance with propofol, it was not possible to achieve the same percentage reduction in CI during simulated haemorrhage before MAP reached the predetermined limit of 40 mm Hg. However, for the induced CI change, there was a reduced vasoconstrictor response. The incidence of an identifiable sudden onset of phase II was not changed, but the duration of phase I was reduced.

Previous studies in humans have suggested that earlier circulatory collapse may occur in humans with combined hypoxia and hypovolaemia [17, 18]. However, as we have found previously in the rabbit, the initial vasoconstrictor response to hypovolaemia was prolonged by hypoxia, although the gradient of the response was unchanged [9]. The effect of midazolam and alfentanil in prolonging phase I of the response to hypovolaemia was similar to that of hypoxia. With propofol infusion and hypoxia, phase I was not prolonged and conductance was increased rather than decreased. The resting vasodilatation after propofol implies that the reflex response to hypovolaemia would have to be greatly enhanced in order to reach the same end-point of vascular conductance. The actual response was reduced in comparison with control and then diverged further from the normal response with hypoxia.

Use of propofol for postoperative sedation may improve regional blood flow and reduce cardiac work because of increased vascular conductance. The present study demonstrates that care should be taken in monitoring circulatory volume and oxygen saturation in that situation because of the decreased effectiveness of reflex circulatory responses. In the absence of continuous monitoring, or in situations where the maintenance of blood volume is difficult, the use of midazolam sedation may be preferable.

Postoperative patients may be rendered liable to the effects of combined hypoxia and hypovolaemia by the delayed elimination of respiratory depressant drugs and by continuing fluid shifts or blood loss resulting from the surgical procedure. The cardiovascular effects of hypoxia include local vascular responses, chemoreceptor-mediated effects, central pressor effects and modification of other reflex responses. In humans, hypovolaemia has been found to increase the chemoreceptor-mediated ventilatory and muscle vasoconstrictor responses to hypoxia [18]. However, anaesthetic agents are potent depressors of cardiovascular reflexes, so a predominance of local vasodilator effects might be anticipated in some anaesthetized or sedated subjects.

Acknowledgements

This work was supported by the National Heart Foundation Ramaciotti Foundations and by Roche Products Ltd. We thank Giunina Joshua and Linda Cornithwaite for technical assistance.

References