Endocrine and metabolic effects of hypotension or halothane inhalation in sheep anaesthetized with pentobarbital

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Summary
We have studied the mechanism whereby halothane induces adrenocortical activity in eight sheep anaesthetized twice for 2 h with pentobarbital. During the second hour they received infusion of nitroprusside to lower mean arterial pressure by 40 mm Hg (group NP) or on another occasion they inhaled 0.5% halothane (group HAL). Plasma concentrations of cortisol, adrenocorticotropic hormone (ACTH), arginine vasopressin (AVP), glucose and lactate were measured. Infusion of nitroprusside increased cortisol concentrations from mean 44 (SD 33) to 256 (131) nmol litre\(^{-1}\) (\(P < 0.05\)) and AVP from 14 (11) to 32 (29) pmol litre\(^{-1}\) (\(P < 0.05\)) while ACTH decreased from 32 (13) to 25 (10) pmol litre\(^{-1}\). The AUC\(_{60–120}\) values for all three hormones were significantly lower in group HAL than in group NP (\(P < 0.01\)). Glucose was unchanged but lactate concentration decreased. These results support, but do not prove, the hypothesis that hypotension is the main stimulant to pituitary–adrenocortical activity during halothane anaesthesia in sheep. A direct effect of nitroprusside cannot be ruled out. (Br. J. Anaesth. 1998; 80: 208–212)

Keywords: anaesthetic techniques, hypotensive; hypnotics barbiturate, pentobarbital; anaesthetics volatile, halothane; hormones, adrenal; metabolism, endocrine; sheep

It has long been recognized that noxious stimuli induce an endocrine stress response in mammals, manifested as increased pituitary–adrenocortical (PAC) activity leading to an increase in circulating cortisol.\(^1\) It is also well known that surgery causes a stress response but anaesthesia itself is generally considered to be relatively benign.\(^2\) Taylor\(^3\) reported that horses had a marked adrenocortical response to volatile agent anaesthesia which is not seen during i.v. anaesthesia with either propofol, barbiturates, detomidine–ketamine or cloramol–ketamine.\(^4\) Sheep have been found to respond to halothane and barbiturate anaesthesia in a manner similar to that of horses\(^5\) and the question arises as to whether or not volatile anaesthesia acts as a noxious stimulus in most species. It is conceivable that in humans, rigorous attention to maintenance of fluid balance and cardiovascular homeostasis during anaesthesia may prevent a stress response from occurring. In addition, as there are few studies in humans where anaesthesia has been investigated in the absence of surgery,\(^6,7\) any effects caused solely by anaesthesia may have been obscured.

It seems likely that some aspect of volatile agent anaesthesia acts as a noxious stimulus inducing a stress response, at least in horses and sheep. It is possible that hypotension and its related effects on perfusion may be responsible. Certainly, haemorrhage is known to cause or enhance adrenocortical activity.\(^8\) However, it is possible that volatile agents may induce an endocrine response by some other unidentified mechanism.

In this study, designed to investigate what stimulates PAC activity during halothane anaesthesia in sheep, an attempt was made to differentiate hypotension from a putative specific effect of halothane. The effects of halothane-free (nitroprusside-induced) hypotension or hypotension-free (sub-anaesthetic doses) halothane exposure were investigated during barbiturate anaesthesia. Pentobarbital was chosen as this does not induce adrenocortical activity.

Materials and methods

ANIMALS

We used eight non-pregnant Welsh Mountain ewes, aged 2–3 yr, weighing 27–39 kg (mean 33 kg) and the study was conducted under Home Office licence PPL 80/83. They were housed indoors with free access to hay and water, except during the 18 h before anaesthesia when they were allowed only water. Each ewe was investigated in two studies, with at least 1 week between anaesthetics. The ewes were prepared for monitoring by surgical exteriorization of the carotid artery into a skin loop at least 2 weeks before the experiment (\(n = 2\)) or by placement of an indwelling polytetrafluoroethylene catheter in the aorta via the femoral artery at least 3 days before the experiment (\(n = 6\)).\(^9\) Preparatory surgery was performed under halothane anaesthesia. For each experiment, a 14-SWG catheter was inserted in the jugular vein and, when appropriate, a 20-SWG catheter was inserted in the relocated carotid artery approximately 30 min before induction of anaesthesia.

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Endocrine response to hypotension

Figure 1  Mean (SD) arterial pressure in eight sheep during pentobarbital anaesthesia. Group NP: nitroprusside-induced hypotension between 60 and 120 min; group HAL: 0.5% inspired halothane between 60 and 120 min. Anaesthesia was induced at 0 min. Pentobarbital infusion was stopped at 120 min. *P < 0.05 compared with control; †P < 0.05 compared with 60-min value. AUC\textsubscript{60–120} was significantly different between groups (P < 0.05).

ANAESTHESIA

Anaesthesia was induced, without premedication, with pentobarbital 16–29 (mean 22) mg kg\(^{-1}\) i.v. and the trachea intubated with a cuffed tracheal tube. Anaesthesia was maintained for 2 h with pentobarbital 0.2–0.3 mg kg\(^{-1}\) min\(^{-1}\) as required, given by incremental doses at 5-min intervals or by infusion (Harvard syringe pump), resulting in a total maintenance dose of 16–39 (mean 31) mg kg\(^{-1}\). The sheep breathed spontaneously throughout anaesthesia and oxygen was supplied either from a circle breathing system or via a T-piece. After 2 h of anaesthesia the sheep were allowed to recover breathing air; the tracheal tube was removed when the swallowing reflex returned.

During the second hour of anaesthesia, sodium nitroprusside was infused to produce hypotension, approximately 40 mm Hg below control values (group NP). A total dose of nitroprusside 405 (162) \(\mu\)g kg\(^{-1}\) was given. On another occasion, during the second hour of anaesthesia, the sheep breathed 0.5% (inspired) halothane in oxygen from a T-piece with fresh gas flow rates sufficient to prevent any rebreathing (group HAL). Four of the sheep received nitroprusside first and halothane second. The other four received halothane first and then nitroprusside.

MEASUREMENTS

The femoral or carotid artery catheter was used to record arterial pressure (Lectromed equipment) and for withdrawal of blood samples for pH and arterial blood-gas measurements (Radiometer 130). Heart rate was obtained from the pressure trace and ventilatory frequency by observation of the chest wall. Venous blood samples (5 ml) were obtained for endocrine and metabolite assays. Blood was divided between tubes containing EDTA and heparin and centrifuged immediately at 4°C; plasma was harvested, frozen and stored at −20°C until hormone and metabolite assay.

Control measurements were made approximately 20 min after the catheters had been inserted, when the sheep had been standing quietly under minimal restraint in sight of another ewe. A continuous arterial pressure trace was recorded for at least 10 min before any other measurements were made and blood samples obtained. During anaesthesia, sampling took place at 20-min intervals; another sample was obtained 20 min after the end of anaesthesia.

Depth of anaesthesia was monitored by normal clinical assessment, and the same range of pentobarbital infusion rates was used in both groups. The total dose of pentobarbital was 55 (6) mg kg\(^{-1}\) in group NP and 53 (4) mg kg\(^{-1}\) in group HAL (P > 0.05). Time to standing was recorded in minutes from the end of the 2-h anaesthetic period when the animals started to breathe air.

BIOCHEMISTRY

Plasma concentrations of cortisol, adrenocorticotrophic hormone (ACTH) and arginine vasopressin (AVP) were measured using radioimmunoassay (RIA).\(^{14,15}\) Intra- and inter-assay coefficients of variation were, respectively, for cortisol 5.6% and 11.8%, for ACTH 8.1% and 16.4% and for AVP 16.8% and 21.5%. Plasma glucose and lactate concentrations were measured using standard colorimetric techniques modified for use on an automatic analyser.

STATISTICAL ANALYSIS

Results are given as mean (SD) unless stated otherwise. Changes with time within any study were analysed using analysis of variance (ANOVA) for repeated measures, followed by Dunnett’s test to examine deviation from control or 60-min values (as indicated) in each anaesthetic study. Differences between groups were analysed by Student’s unpaired \(t\) test of the area under the time curve taken from control to 20 min after anaesthesia (AUC\textsubscript{60–120}) and from 60 to 120 min of anaesthesia (AUC\textsubscript{60–120}).\(^{16}\) Data that were not normally distributed were subjected to log transformation before analysis. \(P < 0.05\) was considered significant.

Results

GROUP NP

Mean arterial pressure (MAP) did not change from control values during the first hour of anaesthesia but during infusion of nitroprusside it decreased significantly (\(P < 0.05\)) from the 60-min level to mean values of 63–68 mm Hg (fig. 1). MAP returned to control values within 20 min of ceasing nitroprusside and pentobarbital administration. Arterial oxygen tension (\(P_{\text{O}_2}\)) increased (\(P < 0.05\)) while the sheep inspired a high oxygen fraction; arterial carbon dioxide tension (\(P_{\text{CO}_2}\)) also increased (\(P < 0.05\)) and pH decreased (\(P < 0.05\)) (table 1). Heart rate and ventilatory frequency did not change significantly (table 1).

Plasma concentrations of cortisol (fig. 2), ACTH (fig. 3) and AVP (fig. 4) did not change significantly during the first hour of anaesthesia but all increased above the 60 min value thereafter (\(P < 0.05\)). Glucose and lactate concentrations did not change significantly throughout anaesthesia (table 1).

All sheep in group NP recovered normally and were standing, eating hay within 120 min after the end of anaesthesia.
AUC60–120 was significantly different between groups (P < 0.01).

Pentobarbital infusion was stopped at 120 min. *P < 0.05 compared with control; †P < 0.05 compared with 60-min value. AUC60–120 was significantly different between groups (P < 0.01).

MAP changed little from control values during the first hour of anaesthesia but decreased below control values during halothane administration to mean values of 89–90 mm Hg (P < 0.05) (fig. 1). The values recorded during halothane inhalation were not significantly lower than the 60-min value (P > 0.05). MAP–AUC60–120 was significantly higher than that recorded in group NP (P < 0.05). MAP returned to control values within 20 min of ceasing halothane administration. PaO₂ and PaCO₂ increased (P < 0.05) during anaesthesia and pH decreased (P < 0.05) (table 1). Heart rate did not change significantly (table 1) and ventilatory frequency decreased only transiently at 20 min. There were no significant differences in arterial blood-gas tensions, heart rate or ventilatory frequency between groups HAL and NP.

There were no significant changes in plasma concentrations of cortisol (fig. 2), ACTH (fig. 3) or AVP (fig. 4) during halothane administration and AUC60–120 for all three hormones was significantly lower than that recorded in group NP (P < 0.01). Glucose concentration did not change but lactate decreased after 60 min, reaching mean values of 0.6 mmol litre⁻¹ at 120 min (P < 0.05) (table 1). There were no significant differences in glucose or lactate concentrations between groups NP and HAL.

Table 1. Mean (sd) arterial oxygen partial pressure (PaO₂), arterial carbon dioxide partial pressure (PaCO₂), pH, heart rate, and plasma glucose and lactate concentrations during pentobarbital anaesthesia in eight sheep with nitroprusside-induced hypotension (group NP) or 0.5% inspired halothane group HAL. Anaesthesia was induced at 0 min. Pentobarbital infusion was stopped at 120 min. Halothane or nitroprusside was given from 60 to 120 min. *P < 0.05 compared with control; †P < 0.05 compared with 60-min value. AUC60–120 was significantly different between groups (P < 0.05).
Endocrine response to hypotension

All sheep in group HAL recovered normally; within 120 min after the end of anaesthesia they were standing and eating hay.

Discussion

We have found that nitroprusside-induced hypotension during pentobarbital anaesthesia caused marked PAC activity in sheep; this supports the hypothesis that during halothane anaesthesia, adrenocortical activity is stimulated by hypotension. However, as it proved impossible to give low doses of halothane without causing slight hypotension, it is not clear if the slight PAC stimulation during halothane administration was caused by mild hypotension or by another effect of halothane. It is also impossible to exclude a direct effect of nitroprusside on PAC activity.

Increases in circulating ACTH, AVP and cortisol have been recognized for some time as integral components of the stress response, although the precise changes evoked depend on the nature of the stressor and the species involved. It has long been known that haemorrhage is one such stressor and that it induces PAC activity through stimulation of afferent input from the baroreceptors. Caraty and colleagues demonstrated that haemorrhage in sheep caused an increase in corticotrophin releasing factor (CRF) and AVP concentrations in hypothalamic portal blood (HPB), with a consequent increase in peripheral concentrations of ACTH and cortisol. Unfortunately, they did not report the simultaneous effects on arterial pressure. Nitroprusside-induced hypotension was used by Keller-Wood and Wood as a stressor in a study of PAC behaviour in conscious sheep; results indicated that hypotension alone induced marked PAC activity. The authors reported large increases in plasma ACTH in response to a slightly greater degree of hypotension than that reported in this article. It is conceivable that pentobarbital anaesthesia may blunt the response to hypotension, but the increases in ACTH, AVP and cortisol were substantial none the less. Most investigations into endocrine responses to induced hypotension in humans have been confined to sympathoadrenal activity and the renin–angiotensin–aldosterone axis. Newton and colleagues studied a wider range of endocrine and metabolic responses to induced hypotension during anaesthesia for middle ear surgery and found that three hypotensive methods led only to a minimal endocrine response typical for “low stress” surgery. Nitroprusside was administered into the HPB in response to stress and acts as one stimulus to ACTH release. However, it is also involved in cardiovascular homeostasis and is released into the peripheral circulation from the posterior pituitary in response to hypotension and increases in blood osmolality. AVP is also released in response to atrial firing when filling pressure is reduced. In our study, the relative contribution of hypotension or decreased filling pressure to stimulation of AVP release was not addressed as filling pressures were not measured. The precise role of AVP as a component of the stress response is unclear; its release in response to hypotension can be regarded as a reflex response to maintain cardiovascular stability. However, AVP is also released in response to stimuli such as surgery where arterial pressure is not affected, the amount released appearing to correlate with the degree of surgical stimulus.

The influence of anaesthesia on AVP release is less clear. In a preliminary study, it was shown that some anaesthetics, including halothane, increased circulating AVP, while others such as pentobarbital had little effect. Simpson and Forsling reported that patients anaesthetized with halothane during bypass surgery developed much higher circulating concentrations of AVP than those receiving nitrous oxide only. In contrast, Leighton, Lim and Wilson showed that halothane anaesthesia does not always affect plasma AVP. There is no doubt that AVP is involved in arterial pressure control during anaesthesia, and is increased by hypotension. However, it is likely that the relationship is complex and depends on fluid load and perfusion, not simply on arterial pressure.

Although this study has not been able to confirm that adrenocortical activity induced during halothane anaesthesia is a result of its hypotensive effect, the data are consistent with the hypothesis. If this proves a correct assumption the question arises as to whether or not PAC activity results from hypotension-induced increases in baroreceptor afferent input or from a more fundamental effect associated with hypotension, such as reduced tissue perfusion. Increases in lactate are commonly reported as a component of the metabolic response to surgery but may also relate to reduction in tissue perfusion and an increase in anaerobic metabolism. Lactate decreased during the second hour of anaesthesia in group HAL but did not change in group NP. This suggests that tissue perfusion may have been worse during nitroprusside administration, presumably as a result of hypotension. Inadequate tissue perfusion may act as, or enhance, the stimulus to the stress response during anaesthesia.

In our study, only the inspired halothane concentration was measured as herbivores exhale a substantial amount of methane which affects the output of infrared anaesthetic agent monitors. However, as no rebreathing took place and all sheep experienced similar rates of fresh gas flow, the amount of halothane received was sufficiently similar for the purposes of this study. No attempt was made to measure cardiac output or any other index of tissue perfusion and there was no evidence that nitroprusside and halothane affected arterial pressure, and thus tissue perfusion, by the same mechanism. Future studies should be directed towards investigating the effect of halothane anaesthesia where normotension is maintained by fluid or inotrope infusion. Inclusion of measures of tissue perfusion would aid interpretation of the data.

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References


