Induction of anaesthesia with halothane and isoflurane in the rabbit: a comparison of the use of a face-mask or an anaesthetic chamber

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Summary

The effects of induction of anaesthesia with halothane or isoflurane were studied in rabbits. The anaesthetic agents were delivered either via a face-mask, or the animals were placed in an anaesthetic induction chamber. All rabbits had periods of apnoea during induction, lasting 30–120 s, resulting in moderate hypercapnia and acidosis. Periods of apnoea were associated with a marked bradycardia. The combination of bradycardia and hypercapnia during induction may represent an increased risk of anaesthetic associated mortality. Animals in all groups tried to avoid inhaling anaesthetic vapour, and this behaviour, together with the occurrence of breath-holding suggests that induction was aversive.

Keywords Rabbit; halothane; isoflurane; breath-holding; bradycardia; anaesthesia

Halothane and isoflurane are widely used for anaesthesia of laboratory species, and both agents can be used successfully in the rabbit (Flecknell 1987). The MAC value of both agents has been established in this species (Drummond 1985) and some data concerning the effects of isoflurane and halothane anaesthesia have been published (Drummond 1986; Sartick 1979; Wyler & Weisser 1972). One advantage of the use of volatile anaesthetics is that animals recover consciousness rapidly, particularly after short periods of anaesthesia. In addition, isoflurane undergoes virtually no biotransformation (Eger 1981), so that following recovery from anaesthesia, alterations in liver microsomal enzyme systems are likely to be minimal.

In small mammals, such as rats and mice, induction of anaesthesia with volatile agents is often carried out in an anaesthetic chamber. In larger species, such as dogs and cats, induction is usually achieved by use of a face-mask. This technique may be resented by the animal, may result in struggling and presumably causes some distress. To avoid this problem in larger species, induction is usually attained using a short-acting injectable agent (e.g. thiopentone or propofol) followed by administration of a volatile agent via an appropriate anaesthetic circuit. When attempting to minimize possible interactions between the anaesthetic regimen used and a particular experimental protocol, it may be advantageous to avoid the use of several different anaesthetic agents, and induce anaesthesia solely with a volatile agent. In rabbits, we have previously considered the use of an anaesthetic chamber inadvisable because of the risk of injury to the animal during the involuntary excitement which can occur during induction (Flecknell 1987). No detailed evaluation of this method has been undertaken, however, and it was considered possible that use of an induction chamber might be better tolerated by rabbits than use of a face-mask.

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The present study was designed to investigate the short-term effects of induction of anaesthesia with halothane or isoflurane, and to compare induction using either a face-mask or an anaesthetic chamber.

Materials and methods

Animals

Young adult female New Zealand White rabbits (body weight 2.7 ± 0.4 g, mean ± 1SD) were obtained from a commercial supplier. The rabbits were free from recognized respiratory pathogens, and were maintained in a unit that obtained all rabbits from colonies which were free from respiratory pathogens. Animals were group housed on dust-free shavings ('Gold Shavings', S.D.S., Witham, Essex, UK) at a stocking density of 6000 cm^2/animal, and fed a commercial pelleted diet *ad libitum* (Rabma pellets, S.D.S., Witham, Essex, UK) and autoclaved hay. Room temperature was maintained at 18 ± 1°C, relative humidity 50%, with 18 air changes/h. Eight rabbits were used, each animal undergoing each of the 4 anaesthetic regimens with an interval of at least 7 days between treatments. The order of treatments was randomized between rabbits. Technical difficulties during induction (failure of catheters and disconnection of ECG cables due to movement of the animal) required that some data be discarded. This resulted in complete records being available for 7 animals in the halothane/chamber group, and 6 animals in each of the other 3 groups.

Apparatus

A commercially-produced anaesthetic induction chamber was used (IMS, Dane Mill, Broadhurst Lane, Congleton, Cheshire CW12 1LA, UK) measuring 45.5 × 30.5 × 30.5 cm. The chamber was constructed of perspex, with a gas inlet port at the base of one end of the chamber, and an outlet for surplus gas at the top of the opposite end. The existing seal on the lid of the chamber was replaced with plasticine, to allow a relatively gas-tight seal around the various sampling cannulae and ECG leads. Three gas sampling cannulae were positioned in the chamber at 5, 15 and 25 cm above the base (designated points a, b and c respectively).

A small rubber conical face-mask (base 8 cm, apex 3 cm, length 11 cm), (Alfred Cox (Surgical) Ltd, Edward Road, Coulsdon, Surrey CR3 2XA, UK) attached to an unmodified Bain's circuit was used for mask induction. The mask was modified to allow two cannulae to be placed at the apex of the mask, close to the fresh gas inlet. One cannula was connected to an MR 11 respiratory monitor (Graseby Dynamics) to monitor respiratory rate. The other cannula was used to sample anaesthetic gas for analysis.

Halothane and isoflurane were delivered from Fluotec III and Isotec III vaporizers respectively (Cyprane, Keighley, Yorks). A fresh gas flow rate of 5 l oxygen/min was delivered to the chamber, and a flow of 2 l/min was delivered via the face-mask. The concentration of anaesthetic agent delivered was measured using a Lamtec agent monitor (Lamtec 601, Lamtec Medical Equipment, England). Electrodes were placed on the skin on the medial aspects of the upper forelegs and left hindleg of the rabbits, and the electrocardiogram recorded using a standard 3-lead system (Grass Polygraph, Grass Instrument Co, Quincy, Mass. USA). Blood pressure was recorded using electronic monitoring apparatus ('Supermon' Kontron Ltd, St Albans, Herts) from a cannula (22G, Intravalve, Ecouen, France) placed in the central ear artery. The rabbit's ears were pre-treated with local anaesthetic (EMLA, Astra Pharmaceutical Ltd, Kings Langley, England) to prevent any pain during placement of the 'over-the-needle' catheter (Flecknell *et al.* 1990). A second catheter was inserted into the other ear artery, to avoid loss of blood pressure data during blood sampling for blood gas analysis. Blood gas analysis was performed using a Stat Profile 3 (Nova Biomedical, Waltham, USA). All catheters and ECG leads were passed through a stockinette bandage, which was applied around the rabbit's shoulders. The Kontron blood pressure module and the polygraph were interfaced to a microcomputer (Elonex PC320X, Elonex, London, England) via a CED 1401 data acquisition module (Cambridge Electronic Design Ltd, Science Park, Milton Road,

Cambridge). This enabled rapid analogue to digital conversion of the blood pressure and ECG signals with continuous data acquisition to the computer hard-disc.

Experiment1 — Anaesthetic chamber characteristics

Prior to commencing any studies involving animals, the filling characteristics of the chamber were determined. A fresh gas flow of 5 l/min was delivered, with a vaporizer setting of either 5% halothane or 5% isoflurane. This resulted in a delivered concentration of 4.3% and 4.7% respectively. Anaesthetic gas concentration at the three sampling points, a, b and c were measured every 30 s for 20 min. Following each trial, the chamber was emptied, the gas concentration measured to ensure no residual anaesthetic remained, and the procedure repeated. Five trials were undertaken with each agent.

Experiment 2—Induction of anaesthesia in chamber

After placement of the ECG leads and arterial catheters as described above, a 5–10 min period was allowed to elapse and the rabbit placed into the chamber. Baseline measurements were taken, and halothane and isoflurane were then delivered at 5 l/min, 4.6 and 4.7% measured concentration. Anaesthetic gas concentrations were measured at 30 s intervals at the 3 sampling points, a, b and c. Arterial blood samples were taken at 2 min intervals until induction was complete, as judged by loss of righting reflex and onset of a regular quiet pattern of respiration. Respiratory rate was counted and recorded, and any periods of apnoea noted and logged directly onto the data acquisition system. The rabbit was observed closely to record behavioural changes during induction. Following induction of anaesthesia, the animal was removed from the chamber, the pedal withdrawal response assessed by pinching the inter-digital skin of one hindlimb, and the arterial lines and ECG leads removed. The animal was observed until it regained its righting reflex, and was then placed in an incubator maintained at 32°C until fully recovered from anaesthesia.

Experiment 3 — Induction of anaesthesia by face-mask

The animals were prepared as described above. After a 5–10 min period, baseline measurements were recorded, following which the animal was restrained manually by holding it gently but firmly and the face-mask applied. More extensive manual restraint was applied when necessary. Halothane and isoflurane were delivered at a rising concentration with 0.5% increments in vaporizer setting every 30 s, rising to a measured, delivered concentration of 4.6% halothane and 4.7% isoflurane. The anaesthetic gas concentration was maintained at this maximum level until induction was complete.

Blood samples were removed for blood gas analysis at 2 min intervals, and respiratory rate counted using the MR respiratory monitor. Periods of apnoea were noted and logged in the data acquisition system. After induction was complete, based on loss of righting reflex, withdrawal reflex, and onset of regular, quiet respiration, anaesthesia was discontinued, the catheter and ECG leads removed, and the animal placed in an incubator as described above.

Statistical methods and data processing

All statistical analyses were performed using the computer program Minitab (ver 8 for PC, Minitab plc). The heart rate and blood pressure data were analysed using Spike 2 (Cambridge Electronic Design Ltd, Science Park, Milton Road, Cambridge). The heart rate and blood pressure at the start of the induction period (t=0) and at the end of anaesthesia were noted. The heart rate and blood pressure traces were inspected for any arrhythmias. The lowest heart rate was noted together with the blood pressure at this time point. The blood gas values at time=0, and the maximum rise in pCO_2 and fall in pO_2 and pH were noted. These values were analysed for the effect of method of anaesthetic delivery (mask or chamber) and anaesthetic agent (halothane or isoflurane), by general linear model (GLM) analysis of variance. To determine whether pCO_2, pO_2, pH, arterial blood pressure and heart rate were affected by anaesthetic induction (irrespective of the type

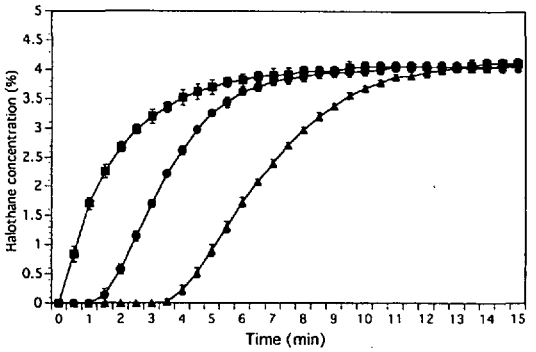

Fig 1 Rate of increase in halothane concentration in anaesthetic chamber (chamber size= 45.5 × 30.5 × 30.5 cm). Fresh gas flow rate, 5 l/min, 4.3% delivered halothane concentration, sampling catheters placed at (a) 5 cm (■), (b) 15 cm (●) and (c) 25 cm (▲) above the base of the chamber

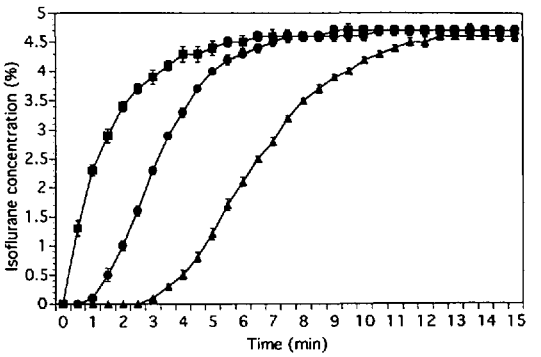

Fig 2 Rate of increase in isoflurane concentration in anaesthetic chamber (chamber size= 45.5 × 30.5 × 30.5 cm). Fresh flow rate, 5 l/min, 4.7% delivered isoflurane concentration, sampling catheters placed at (a) 5 cm (■), (b) 15 cm (●) and (c) 25 cm (▲) above the base of the chamber

of delivery system or agent used), Student's paired *t*-test was used to compare differences between baseline measurements and those at peak effect and at the end of anaesthesia.

Results

Experiment 1

Figs 1 and 2 show the anaesthetic gas concentrations at the various levels in the anaesthetic chamber for halothane and isoflurane. Both anaesthetic gases filled the chamber in a graduated manner. High concentrations of anaesthetic were rapidly attained at the lowest point in the chamber (a), and during this period concentrations at the upper levels (b and c) were low or undetectable. Concentrations then increased in the middle layer of the chamber (b), followed finally by the upper layer (c).

Experiment 2

The chamber filling pattern was very similar to that noted in Experiment 1. Small variations occurred during movement of the animal, but these had remarkably little effect on the gas concentrations. It was only on a few occasions that marked excitement and struggling during induction resulted in significant mixing of gas (Fig 3).

All rabbits behaved in a very characteristic fashion. Initially, the animals remained in a normal posture in the chamber, or explored the chamber walls. All rabbits were tachypnoiec (238 ± 59 bpm, halothane; 211 ± 56 bpm, isoflurane). Immediately halothane or moderate concentrations (>0.5%) concentrations of isoflurane were attained at the level of the animal's nose, the rabbit raised its head and pressed its nose into one of the top corners of the chamber. The rabbits remained in this position for a variable period of time, generally 1–2 min, following which they lowered their heads into the anaesthetic vapour. All animals then ceased respiration for a period of between 30 and 120 s. Intermittent periods of breathing were interspersed with further periods of apnoea, until the animal began to lose consciousness, at which time a normal respiratory pattern returned. During this period, most animals pawed at their nose and face, and made violent attempts to escape from the chamber. Two rabbits which received halothane and one which received isoflurane vocalized while making forceful expiratory movements after losing their righting reflex. The changes in cardiovascular parameters are summarized in Table 1. During apnoeic periods, marked bradycardia was noted. This was followed by a pronounced tachycardia as normal ventilation and loss of consciousness occurred. The effects of apnoea on blood gas parameters are shown in Table 2. Rabbits in both groups developed hypercapnia and acidosis.

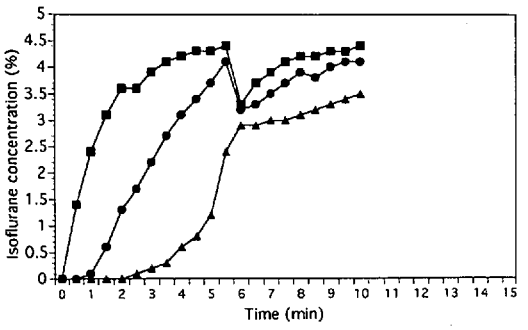

Figure 3. Rate of increase in isoflurane concentration in anaesthetic chamber (chamber size= 45.5 × 30.5 × 30.5 cm) during induction of anaesthesia of rabbit. Fresh gas flow rate, 5 l/min, 4.7% delivered isoflurane concentration, sampling catheters placed at (a) 5 cm (■), (b) 15 cm (●) and (c) 25 cm (▲), above the base of the chamber. The animal struggled and showed marked involuntary excitement at t=6

Experiment 3

All rabbits were tachypnoeic before induction (223 ± 69 bpm, halothane; 208 ± 46 bpm, isoflurane). Immediately halothane was introduced via the face-mask, all rabbits became apnoeic. After a variable period (30–120 s) a breath was taken, followed by further periods of apnoea. All animals attempted to escape from their restraint, either immediately following first exposure to the anaesthetic vapour, or following an initial period of apnoea. Eventually a normal respiratory pattern was re-established as the animal lost consciousness. Cardiovascular responses to apnoea were similar to those noted in Experiment 2 (Table 1), as were the changes in blood gas parameters (Table 2). Rabbits inhaling isoflurane continued normal respiration until the inspired concentration rose above 0.5%. At this point, apnoea occurred in all animals, followed by a pattern of intermittent breathing and further periods of apnoea. As with halothane induction, all animals struggled and attempted to escape from their restraint. Once the animals lost consciousness regular respiration recommenced. One rabbit in each group vocalized and made forceful expiratory movements after losing its righting reflex.

Statistical analysis: comparison of effects of induction agent and method of induction

All groups of rabbits had a significant ($P<0.005$) rise in arterial pCO_2 and fall in arterial pH. All groups had a significant ($P<0.005$) reduction in heart rate, and showed a small, non-significant fall in blood pressure ($p<0.05$). Arterial pCO_2 was significantly higher at time=0 in the groups of rabbits anaesthetized using a face-mask ($P<0.001$); however, the difference between peak pCO_2 and that at time=0 did not differ significantly between groups ($P<0.05$). The pre-induction respiratory rate was not significantly different between groups. The method of anaesthetic delivery (mask or

Table 1 Changes in heart rate and blood pressure associated with induction of anaesthesia with halothane or isoflurane in rabbits (values are means)

	Heart rate (bpm)			Mean arterial blood pressure (mmHg)		
	t=0	t=low	t=end	t=0	t=low	t=end
Halothane via chamber, n=7	231	81	291	80	85	73
Isoflurane via chamber, n=6	229	70	236	82	90	81
Halothane via face-mask, n=6	260	48	305	80	69	60
Isoflurane via face-mask, n=6	240	107	241	75	84	65
Pooled s.d.	32	42	54	7	17	17

Data at t=0 were recorded immediately before the onset of induction. Heart rate t=low are the lowest values recorded during induction. Blood pressure t=low is the mean arterial pressure at the lowest heart rate. Data at t=end were recorded after the onset of surgical anaesthesia. Pooled s.d. are the pooled standard deviations for all treatment groups

Table 2 Changes in arterial pCO_2, pO_2 and pH associated with induction of anaesthesia with halothane or isoflurane in rabbits (values are means)

	Arterial pCO_2 (KPa)		Arterial pO_2 (KPa)		Aterial pH (units)	
	t=0	t=high	t=0	t=low	t=0	t=low
Halothane via chamber, n=7	4.1	6.8	13.2	11.9	7.386	7.2241
Isoflurane via chamber, n=6	4.1	7.2	13.3	12.4	7.3967	7.2062
Halothane via face-mask, n=6	4.9	8.7	13.2	12.3	7.4005	7.1587
Isoflurane via face-mask, n=6	4.9	8.3	14.4	13.6	7.4033	7.2305
Pooled s.d.	0.6	1.3	1.1	2	0.0267	0.0653

Data at t=0 were recorded immediately before the onset of induction. Data at t=low and t=high are the maximum changes from baseline recorded during induction. Pooled s.d. are the pooled standard deviations for all treatment groups

chamber) had no significant effect on heart rate, pO_2, pH or blood pressure at $t=0$. Blood pressure was significantly lower ($P<0.05$) at the end of anaesthesia in animals induced using a face-mask. The agent used (halothane or isoflurane) had no significant ($P<0.05$) effect on any of the variables studied, with the exception of the heart rate at the end of the period of anaesthesia, which was significantly higher in rabbits receiving halothane.

Discussion

The filling characteristics of the chamber could perhaps be predicted from the density of the anaesthetic gases (mol wt. halothane=197.39; isoflurane=184.57) but it should be noted that the jet-effect from the inlet had no effect in disturbing the layer of anaesthetic vapour which gradually developed in the chamber. It was anticipated that movements of the animal would produce a mixing effect, but this did not occur to any significant extent in most of the trials. This stratification of vapour often resulted in rabbits inhaling very high concentrations of halothane or isoflurane, rather than being exposed to a gradually rising concentration of the agent.

All rabbits had tachypnoea, when compared to respiratory rates recorded in our laboratory in undisturbed resting rabbits by observation via a viewing panel (59 ± 9, $n=9$). Tachypnoea is frequently observed in rabbits following removal from their cage or pen and exposure to less familiar surroundings. The pre-existing tachypnoea resulted in a mild hypocapnia, which was significantly greater in animals placed in the anaesthetic chamber. This may have arisen because of a slightly greater time-delay between removal of these groups of animals from their transport box, placement in the chamber, and baseline blood gas measurements. To avoid the lower baseline carbon dioxide tensions in the chamber groups influencing between-group comparisons, the difference between pre-anaesthetic and peak pCO_2 values were compared.

Since the duration of induction varied depending upon the duration and severity of apnoea, it was more useful to compare the maximal effects of induction on heart rate, blood pressure and blood gas parameters, rather than to attempt comparisons at specific time points.

When planning this study, the protocol for face-mask induction was derived from standard veterinary anaesthetic methodology, with the anaesthetic concentration being gradually raised. Because of the occurrence of periods of apnoea, this gradual effect was not achieved. It should be noted that because of the physical restraint employed to avoid risk of injury to the animal should it struggle, it would not normally have been possible to detect these periods of apnoea. Detection of apnoea during induction would only be likely if a respiratory monitor was employed. A rebreathing bag attached to the end of the Bain's circuit might also enable this, but the low tidal volumes of rabbits (20–30 ml) make this method unreliable. In practical terms, a similar situation occurred both in rabbits given anaesthetic by face-mask and those

placed in the chamber. After a period of apnoea, associated with bradycardia and hypercapnia, the animal inhaled a high concentration of the anaesthetic. These results indicate 2 areas of concern: whether the techniques used cause significant distress to animals, and whether the cardiovascular and respiratory effects represent an increased risk of anaesthetic-associated mortality.

In healthy humans, rapid induction with high concentrations of halothane (4%) has been claimed to be safe and effective (Ruffle *et al.* 1985), although the established view has been to increase anaesthetic concentrations gradually. The human volunteers in Ruffle *et al.*'s study were apparently not distressed by the procedure, and did not become hypercapnic. In contrast, it is reasonable to presume that some degree of distress was caused to rabbits in our study, since they were fully conscious and chose to breath-hold for periods of up to 2 min. In addition many animals pawed at their face and nose, and made violent attempts to remove the mask or escape from the chamber after a period of apnoea. The hypercapnia which occurred as a consequence of the breath-holding response may increase the risk of cardiac arrhythmias associated with induction, particularly with halothane, since this agent is known to sensitize the myocardium to the effects of catecholamines (Smith 1990). Although catecholamine concentrations were not measured, it does not seem unreasonable to suggest that they would have been elevated both as a result of hypercapnia, the handling and restraint needed during induction, and exposure to the anaesthetic agent. Induction with a volatile anaesthetic may represent a significantly increased risk of cardiac arrest, and may explain some of the anecdotal accounts of high anaesthetic mortality in rabbits during induction with halothane. However, no obvious arrhythmias were found on inspection of the ECG and blood pressure recordings in this series of rabbits. The vocalization and marked expiratory efforts which occurred in a minority of rabbits occurred after loss of consciousness, and were probably caused by laryngospasm. This has been reported in children following face-mask induction with isoflurane (Pandit *et al.* 1985), and may also contribute to an increased risk of anaesthetic mortality during induction with volatile anaesthetics in rabbits.

The mechanism responsible for the bradycardia which was seen in all animals during periods of apnoea appears similar to that associated with breath-holding in man (Bjurstrom & Schoene 1987; Ferrigno *et al.* 1986). Breath-holding at large lung volumes reduces cardiac output, presumably because of an impairment of venous return (Ferrigno *et al.* 1986) and reduces heart rate by 20–30% in man (Bjurstrom & Schoene 1987). The fall in heart rate in the rabbits in the present study was considerably greater (55–82%).

It might have been predicted that all rabbits would develop mild to moderate hypotension after induction of anaesthesia, since both halothane and isoflurane have been shown to reduce blood pressure in rabbits (Sartick 1979, Wyler & Weisser 1972). The lack of any significant effect in these experiments is probably due to the moderate hypercapnia which occurred, and a possible increase in circulating catecholamines caused both by hypercapnia and by the stress of induction. Although no animals became hypoxic, arterial pO_2 remained low despite using 100% oxygen as the carrier gas.

Induction of anaesthesia in the rabbit with halothane or isoflurane results in apnoea and bradycardia and this may represent an increased risk of death during induction. The behaviour of rabbits suggested that they resented or were distressed by the procedure. It is possible that the use of appropriate pre-anaesthetic medication, or use of a short-acting induction agent might prevent these responses. We suggest that induction of anaesthesia using solely isoflurane or halothane should only be carried out when specifically required in a research protocol.

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