

Further Proof that the Spinal Cord, and Not the Brain, Mediates the Immobility Produced by Inhaled Anesthetics

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Background: Previous investigations indicate that the spinal cord, perhaps with a minor cerebral contribution, mediates the capacity of inhaled anesthetics to produce immobility in the face of noxious stimulation. The implications of these investigations may be limited by the trauma associated with their experimental methods (e.g., cardiopulmonary bypass or transection of the spinal cord). The present study avoided such trauma.

Methods: Thirty goats received emulsified isoflurane *via* either the initial section of the aorta (arterial group; preferential isoflurane delivery to the spinal cord) or an ear vein (venous group; equal delivery of isoflurane to the cord and brain). The authors determined the minimum partial pressure of isoflurane (the isoflurane partial pressure in the blood required to produce immobility in 50% of the goats exposed to a noxious stimulus).

Results: For the venous group, the minimum partial pressure in carotid *versus* femoral arterial blood (9.56 ± 1.86 mmHg *vs.* 9.68 ± 1.90 mmHg) did not differ. For the arterial group, the minimum partial pressure in carotid arterial blood was half that in femoral arterial blood (5.35 ± 1.45 mmHg *vs.* 10.97 ± 3.04 mmHg, $P < 0.05$). As these data show, the minimum partial pressure in femoral arterial blood did not differ for the arterial group *versus* the venous group.

Conclusions: In this novel and minimally traumatic model, the anesthetic partial pressure delivered to the spinal cord governed the suppression of movement in response to noxious stimulation. The results indicate that the spinal cord is the primary mediator of immobility and that the brain plays little or no role.

ALTHOUGH volatile anesthetics have been widely used in surgical operations since the mid 19th century, the action sites and mechanisms of the drugs are still being explored.¹ Some studies indicate that the spinal cord is the primary immobilizing site of anesthetics, and that the brain plays a secondary role.^{2,3} However, the models used in these studies imposed stressful or traumatic changes in the circulation or the central nervous system (e.g., cardiopulmonary

bypass, spinal cord transection), changes that might have confounded the outcomes found.³⁻⁶ A model with an intact circulation and central nervous system would be preferred for such studies. Emulsified isoflurane can be administered directly into the circulation, washed out *via* the lungs, and produces anesthesia by the same mechanism as inhaled isoflurane.⁷⁻⁹ It has also been demonstrated that there are few collateral vessels between the cerebral and the spinal cord circulation in goats.^{10,11} On the basis of these findings, we assumed that a model of preferential delivery of isoflurane to the spinal cord with an intact circulation and central nervous system could be developed by infusing emulsified isoflurane into the initial section of the aorta in goats. The current study was carried out to verify this prediction and to further assess the roles of the spinal cord and the brain as the sites for isoflurane immobilizing action.

Materials and Methods

Emulsified isoflurane used in the current study was manufactured by Huarui Pharmacy, Ltd. (Wuxi, Jiangsu, China) according to the formula developed by our laboratory.^{8,12} In this formula, 1.6 ml of liquid isoflurane (Abbott Laboratories, Queenborough, Kent, United Kingdom) and 18.4 ml of Intralipid® (30%, a lipid emulsion injection, w/v; Huarui Pharmacy, Ltd.) were stored in a 20-ml glass ampoule using an aseptic technique.

Animal Preparation

After obtaining approval from the Institutional Animal Care and Use Committee of Sichuan University (Chengdu, Sichuan, China), 30 goats aged 1-2 yrs and weighing 20.72 ± 1.24 kg (mean \pm SD), were divided into venous group and arterial group. Emulsified isoflurane was administered *via* the initial section of the aorta in the arterial group or an ear vein in the venous group.

The goat was anesthetized with IV injection of 5-8 mg/kg propofol and 1-2 mg/kg suxamethonium. After the palpebral reflex disappeared, an 8-French cuffed endotracheal tube coating with 1% dicitaine gel (Nanjin Pharmacy Ltd., Nanjin, China) for endotracheal topical anesthesia was inserted.¹³ Ventilator settings were adjusted to maintain the end-tidal carbon dioxide partial pressure in the range of 25-35 mmHg. Isoflurane (1.5-2.5%) was used to maintain anesthesia. The heart rate, mean arterial pressure, electrocardiograph, lingual pulse oxygen saturation, and the end-tidal isoflurane concentration were measured by a 150B3 monitor (Philips, Suzhou, China). The rectal temperature

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was measured and maintained at 38–40°C by using a heating lamp and blankets as needed. A peripheral vein of the ear was chosen for infusing lactated Ringer solution ($10 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$). The jugular vein, carotid artery, femoral vein, and femoral artery each were cannulated to permit blood sampling (catheter diameter, 1.1 mm). In the arterial group, a 100-cm-long polyethylene catheter (diameter, 1.7 mm) was inserted from the other femoral artery into the initial section of the aorta for emulsified isoflurane infusion (fig. 1). The positioning of the catheter tip was guided by a 2D ultrasound system (RtFino; General Electric Co., Fairfield, CT) with a probe placed on the left third intercostal space. After the catheter tip was visualized at the level of the aortic valve, the catheter was withdrawn 10 cm, and the tip was just at the initial section of the aorta, as confirmed by an autopsy after experiment.

The goats in both groups were restrained in a straight position with suspended slings, and isoflurane inhalation was immediately stopped. After about 10 min, all goats awoke as indicated by the recovery of palpebral reflex, blood samples (8 ml each) were simultaneously drawn from the femoral artery, femoral vein, carotid artery, and jugular vein. Blood sampling was taken within 1 min, and the endotracheal tube was tolerated well in all the animals due to endotracheal topical anesthesia. The partial pressures of isoflurane (P_{iso}) in the samples were determined using a gas chromatograph (see P_{iso} determination by a gas chromatograph), and the data at this point were accepted as the basal P_{iso} . Emulsified isoflurane was infused with a microinfusion pump immediately after the blood sample collection in both groups. In the arterial group, emulsified isoflurane was infused through the aortic catheter. To avoid isoflurane rebreathing, a high fresh oxygen flow rate ($> 7 \text{ l/min}$) was maintained for ventilation, and the anesthetic absorber was used in the inspiratory circuit. In the venous group, emulsified isoflurane was infused *via* an ear vein, and a low fresh oxygen flow rate (2 l/min) was applied for ventilation.

MAC and MPP Determination

The Dixon up-and-down method and the tail clamping stimulus were used to determine minimum alveolar concentration (MAC) and minimum partial pressure (MPP) of isoflurane (P_{iso} in the alveolar gas or the blood required to produce immobility in 50% of the goats exposed to a noxious stimulus), which represented anesthetic requirements.^{14–16} The initial end-tidal concentration of isoflurane used for MAC determination was maintained for 15 min at about 1 MAC (0.7% for the arterial group and 1.0% for the venous group based on the results of our pilot study) by adjusting the infusion rate of emulsified isoflurane (2–3 $\text{ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for the arterial group and 1–2 $\text{ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for the venous group). Before tail clamping, blood samples (8 ml each) from the femoral artery, femoral vein, carotid artery, and jugular vein were drawn simultaneously. The P_{iso} in the samples was determined using a gas chromatograph.

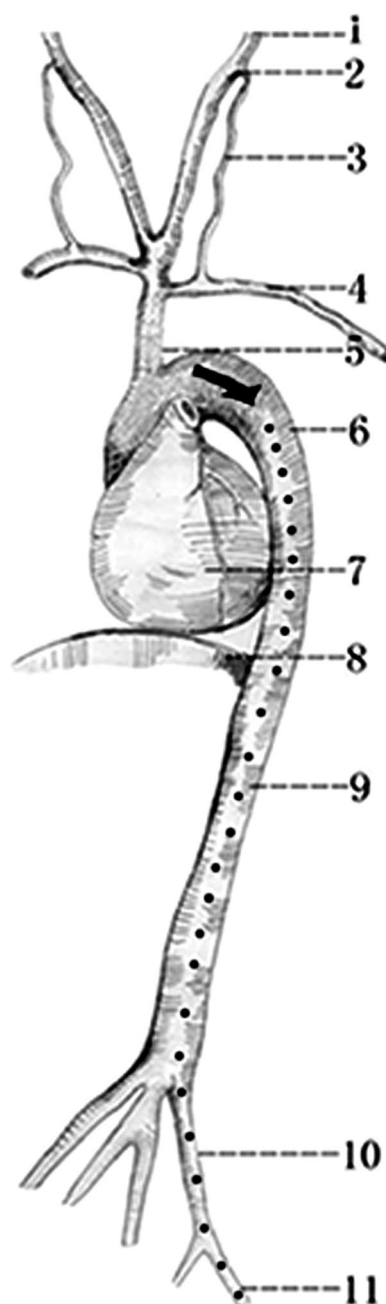


Fig. 1. Vascular anatomy of the goat (ventral aspect). In the current study, a catheter (black dashed line) was inserted from the femoral artery to the initial section of the aorta for emulsified isoflurane infusion. 1 = carotid artery; 2 = occipital artery; 3 = vertebral artery; 4 = subclavian artery; 5 = brachiocephalic artery; 6 = thoracic aorta; 7 = heart; 8 = diaphragm; 9 = abdominal aorta; 10 = left iliac external artery; 11 = left femoral artery.

Then, a pair of forceps was clamped on the shaved tail approximately 5–10 cm from its base in intervals of three times every 15 s for 1 min. A gross purposeful muscular movement was considered to be a positive response to tail clamping.^{3,9} If the response to the tail clamping was positive or negative in the first goat, the end-tidal isoflurane concentration was increased or decreased by 0.2% and maintained for 15 min in the next goat before clamping. The above procedure was repeated until 6 interindividual

Table 1. Comparisons of the Vital Signs at Different Time Points Between the Arterial and Venous Groups

	After Induction	After Inserting Catheters	After Changing Position	Before Tail Clamping	After Tail Clamping	End of Study	P Value
HR, beat/min							
Arterial group	96.3 ± 2.4	95.8 ± 4.8	100.8 ± 4.7	100.4 ± 8.8	101.4 ± 4.7	110.6 ± 5.7	0.97
Venous group	97.8 ± 2.5	94.8 ± 4.2	99.7 ± 5.2	101.0 ± 3.9	100.3 ± 4.9	109.8 ± 5.6	
MBP, mmHg							
Arterial group	87.6 ± 2.4	88.5 ± 3.5	86.9 ± 3.4	87.5 ± 4.2	88.8 ± 5.8	96.2 ± 7.1	0.80
Venous group	87.1 ± 3.3	87.0 ± 2.0	86.9 ± 2.8	88.5 ± 2.3	89.3 ± 6.3	95.0 ± 7.1	

Data are presented as mean ± SD.

HR = heart rate; MBP = mean blood pressure.

crossovers of positive responses *versus* negative responses had occurred, and the averaged 6 midpoint values of P_{50} in the end-tidal gas or in the blood were taken as MAC or MPP.

Administration of emulsified isoflurane was stopped immediately after the last tail clamping. The goats in the two groups were mechanically ventilated with 7 l/min fresh oxygen to wash out isoflurane quickly. All goats completely awakened after about 8 min and were extubated. They were observed for 3 days for possible complications.

P₅₀ Determination by a Gas Chromatograph

The isoflurane concentration in the blood (C) and the determined value of isoflurane blood/gas partition coefficient ($\lambda_{b/g}$) were measured using a gas chromatograph (Agilent 4890D; Tegment Technology Ltd., Hong Kong, China) with the two-stage headspace equilibration method, as previously described.⁹ P_{50} in the blood was calculated by the equation of $P_{50} = (C/\lambda_{b/g} \times 760 \text{ mmHg})$. A technician who didn't know the sources of the samples measured the isoflurane concentrations and the P_{50} of all samples.

Statistics

Data were presented as mean ± SD, and all analyses were performed using SPSS 13.0 (SPSS Inc, Chicago, IL). Comparisons of weight and gender between the two groups were performed by the Student *t* test and the Fisher exact test, respectively. The vital signs between the two groups were compared using a repeated-measures analysis of variance. A one-way analysis of variance was applied to the comparisons of the different P_{50} at each end-tidal isoflurane concentration level. Intragroup differences of the basal P_{50} and MPP were analyzed by a two-way analysis of variance, and the Student-Newman-Keuls test was used for *post hoc* comparisons. Intergroup differences of the basal P_{50} and MPP were analyzed by a one-way analysis of variance. $P < 0.05$ was considered statistically significant.

Results

Thirty goats were used in this study (16 in the arterial group and 14 in the venous group). No significant differences were found in the weight ($20.6 \pm 2.4 \text{ kg}$ *vs.* $20.7 \pm 2.2 \text{ kg}$), gender (female/male, 12/4 *vs.* 9/5), the vital signs,

and the basal P_{50} between the two groups (tables 1, 2). During emulsified isoflurane infusion, none of the goats showed any signs of being awake, as indicated by the lack of head movement, chewing, swallowing, or eye opening. All goats completely awakened approximately 8 min after stopping emulsified isoflurane administration, and no fatalities occurred in the two groups during the 3-day observation after MAC determination.

The responses of the goats to tail clamping during MAC determination are shown in figure 2. The MAC of the arterial group ($0.57 \pm 0.15\%$) was significantly lower than that of the venous group ($1.13 \pm 0.08\%$, $P < 0.05$). At all end-tidal isoflurane concentration levels, the P_{50} of carotid artery was almost half the P_{50} of femoral artery in the arterial group ($P < 0.05$), and the P_{50} of carotid artery and femoral artery were not significantly different in the venous group (table 2). In the arterial group, the MPP of carotid artery was about half the MPP of femoral artery ($5.35 \pm 1.45 \text{ mmHg}$ *vs.* $10.97 \pm 3.04 \text{ mmHg}$, $P < 0.05$), and the MPP of femoral artery showed no significant differences from the MPP of femoral artery in the venous group. In the venous group, the MPP of femoral artery ($9.56 \pm 1.86 \text{ mmHg}$) was not significantly different from the MPP of carotid artery ($9.68 \pm 1.90 \text{ mmHg}$, table 3).

Discussion

In general, the arterial P_{50} is presumed to be in equilibrium with the P_{50} in the central nervous system under steady state conditions.¹⁵ Therefore, the P_{50} of femoral and the carotid artery act as measures for the P_{50} of the spinal cord and the brain, respectively. In the current study, the P_{50} of femoral artery was almost twice that of carotid artery during the aortic delivery of emulsified isoflurane, suggesting that the P_{50} in the spinal cord was about twice the P_{50} in the brain and that isoflurane was preferentially delivered to the spinal cord. All goats completely awakened about 8 min after the termination of emulsified isoflurane infusion, and no fatalities occurred in the two groups, demonstrating a reversible anesthetic action produced by emulsified isoflurane infusion with no apparent pathologic injury in the central nervous system, as well as the safety of emulsified isoflurane infusion (including *via* the aorta).

Table 2. Comparisons of the Different P_{ISO} at Each End-tidal Isoflurane Concentration Level During MAC Determination in the Arterial and Venous Groups

Number of Goats	End-tidal Isoflurane Concentration (%)	P _{ISO} of Carotid Artery (mmHg)	P _{ISO} of Jugular Vein (mmHg)	P _{ISO} of Femoral Artery (mmHg)	P _{ISO} of Femoral Vein (mmHg)	P _{ISO} of Femoral Artery/ P _{ISO} of Carotid Artery
Arterial group						
16	Basal P _{ISO}	1.03 ± 0.07	1.00 ± 0.75	1.02 ± 0.86	0.99 ± 0.06	0.99
		1.00 ± 0.75				
		1.02 ± 0.86				
		0.99 ± 0.06				
1	0.9	7.10	7.02	14.57	13.18	1.87
5	0.7	6.41 ± 0.86*	5.99 ± 0.68*	13.26 ± 1.88	11.40 ± 1.75	2.07
7	0.5	5.28 ± 0.85*	4.74 ± 0.85*	10.51 ± 1.90	9.00 ± 1.17	1.99
3	0.3	2.54 ± 0.43*	2.25 ± 0.17*	5.60 ± 1.06	4.90 ± 0.47	2.20
Venous group						
14	Basal P _{ISO}	1.03 ± 0.09	0.97 ± 0.08	1.00 ± 0.09	0.97 ± 0.07	1.00
1	1.4	12.99	11.53	12.36	11.24	0.95
7	1.2	10.54 ± 1.97	9.74 ± 1.83	10.35 ± 1.89	9.50 ± 1.44	0.98
6	1.0	8.07 ± 1.06	8.16 ± 1.64	8.13 ± 1.19	7.99 ± 1.21	1.01

Data are presented as mean ± SD. Arterial group: emulsified isoflurane was administered *via* the initial section of the aorta. Venous group: emulsified isoflurane was administered *via* a peripheral vein of the ear.

* $P < 0.05$ vs. P_{ISO} of femoral artery and vein at the same end-tidal isoflurane concentration level.

MAC = minimum alveolar concentration; P_{ISO} = the isoflurane partial pressure.

This model is helpful to further elucidate the immobilizing effects of isoflurane at the brain and the spinal cord. In the current study, no significant difference was found between the MPP of carotid artery and femoral artery in the venous group. In contrast, the MPP of carotid artery in the arterial group was only half the MPP of carotid artery in the venous group, but the MPP of femoral artery in the both group was not significantly different. This suggests that immobility does not depend on the partial pressure of isoflurane in the brain and that the spinal cord is the primary site mediating the immobility produced by isoflurane. Our results are consistent with previous studies,^{3,17} which showed that anesthetic immobilizing requirements increase twofold or fourfold

when isoflurane or halothane are selectively delivered to the brain rather than to the brain and body with bypass. Our results are also consistent with those of Rampil *et al.*² that decerebration in rats appears to have little effect on MAC needed to produce immobility. Some studies imply that the brain might mediate a minor part of the immobility produced by inhaled anesthetics.¹⁸⁻²⁰ In contrast, our results do not indicate any contribution of the brain when P_{ISO} in the brain varied from 5.35 ± 1.61 mmHg (the MPP of carotid artery in arterial group) to 9.68 ± 2.28 mmHg (the MPP of carotid artery in venous group), because within this range the MPPs of femoral artery in the two groups are not significantly different.

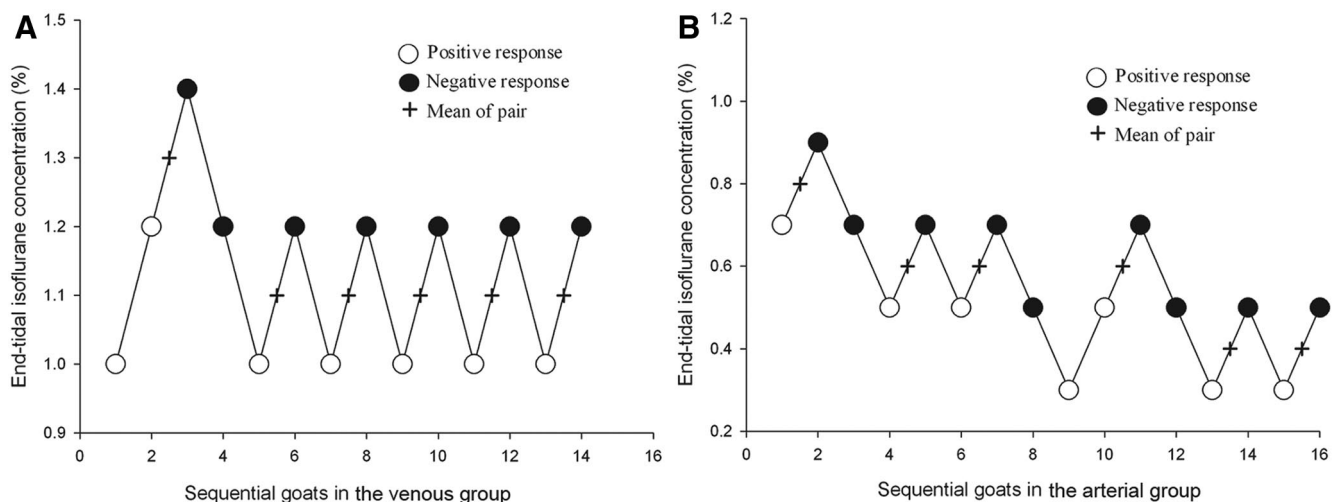


Fig. 2. A and B show the response of the goats to the tail clamping during minimum alveolar concentration (MAC) determination. Data from each goat are represented by a circle. To obtain 6 successive interindividual crossovers, 16 and 14 goats were required in the arterial and venous groups, respectively. The MAC of the venous and arterial groups were $1.13 \pm 0.08\%$ and $0.57 \pm 0.15\%$ (mean ± SD), respectively. Arterial group, emulsified isoflurane was administered *via* the initial section of the aorta in this group; venous group, emulsified isoflurane was administered *via* a peripheral vein of the ear in this group.

Table 3. Intragroup and Intergroup Comparisons Among Different MPP and MAC

	MAC (%)	MPP of Carotid Artery (mmHg)	MPP of Jugular Vein (mmHg)	MPP of Femoral Artery (mmHg)	MPP of Femoral Vein (mmHg)	MPP of Femoral Artery/ MPP of Carotid Artery
Arterial group	0.57 ± 0.15†	5.35 ± 1.45*	4.91 ± 1.46*	10.97 ± 3.04	9.54 ± 2.52	2.05
Venous group	1.13 ± 0.08	9.68 ± 1.90	9.03 ± 1.80	9.56 ± 1.86	8.97 ± 1.60	0.99
Values in the arterial group/ values in the venous group	0.50	0.55	0.54	1.15	1.06	
P value	0.00	0.00	0.00	0.35	0.65	

Data are presented as mean ± SD. Arterial group: emulsified isoflurane was administered *via* the initial section of the aorta. Venous group: emulsified isoflurane was administered *via* a peripheral vein of the ear.

* $P < 0.05$ vs. MPP of femoral artery and vein in the arterial group. † $P < 0.05$ vs. MAC of the venous group.

MAC = minimum alveolar concentration; MPP = the isoflurane partial pressure required to produce immobility in 50% of the goats exposed to a noxious stimulus.

Prevention of animal movements under tracheal intubation in the absence of noxious stimulation is very important in studying the effects of anesthetics on the spinal cord. A previous study has shown preferential delivery of isoflurane to the spinal cord, with a low isoflurane concentration in the brain (0.2–0.3%) with bypass, was ethically or practically difficult because the animal might move constantly and such movement would disrupt the experiment.¹⁸ To prevent possible movement, Antognini *et al.*⁵ delivered 0.5% halothane (0.7 MAC) to the cranial circulation with bypass when examining *o*-difluorobenzene requirements in the model of preferentially anesthetized spinal cord. In the current study with the tracheal topical anesthesia and low P_{iSO} in the carotid artery, we successfully prevented animal movements during the whole study. None of the goats in both groups moved under tracheal intubation in the absence of noxious stimulation, including the three goats in the arterial group with the lowest P_{iSO} of carotid artery (2.54 ± 0.43 mmHg, table 2), which was about one-fourth the MPP of carotid artery (9.68 ± 2.28 mmHg) in the venous group (table 3).

A limitation of the current approach is that it does not separate the cerebral partial pressures of isoflurane as much as one might like (one-fourth the MPP of carotid artery in the venous group with tracheal topical anesthesia preventing movements has been shown in this study). The P_{iSO} for the aortic infusion is still half the P_{iSO} for the intravenous infusion. The limitation is due to the clearance of isoflurane at the lungs limited by its solubility. A greater separation could be achieved by infusing emulsified sevoflurane or desflurane (with low blood-gas solubilities) and concurrent administration of carbon dioxide.²¹

In conclusion, we have developed a novel model to preferentially deliver emulsified isoflurane to the *in situ* goat spinal cord with an intact circulation and central nervous system. Using this model, we confirmed that the spinal cord, and not the brain, is the primary site mediating the capacity of isoflurane to produce immobility.

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