A comparison of the area of histochemical dysfunction after focal cerebral ischaemia during anaesthesia with isoflurane and halothane in the rat

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The investigations that have thus far evaluated the cerebral protective properties of isoflurane have provided conflicting results. Protection would be most likely to occur in the circumstances of incomplete cerebral ischaemia in which there is a penumbral zone of marginal perfusion. The present investigation sought to evaluate further the protective properties of isoflurane in that circumstance. Middle cerebral artery occlusion (MCAO) was performed in Sprague-Dawley rats anaesthetized with 1.2 MAC concentrations of either halothane or isoflurane. At the end of four hours of MCAO, the brains were removed, sectioned and incubated in the histochemical stain 2-3-triphenyltetrazolium (TTC). An image analysis system was used to measure the area of reduced or absent TTC staining in four coronal planes spanning the distribution of the middle cerebral artery. There was no difference between halothane and isoflurane anaesthetized animals with respect to the area of brain with evidence of histochemical dysfunction. It is con-

cluded that isoflurane is not protective (relative to the status of halothane anaesthetized control animals) when administered at 1.2 MAC concentration during four hours of focal (incomplete) cerebral ischaemia in the rat.

Les investigations faites à date pour évaluer les propriétés protectrices cérébrales de l'isoflurane ont fourni des résultats divergents. La protection surviendrait le plus probablement dans les circonstances où l'ischémie cérébrale est incomplète et qui serait accompagnée en périphérie d'une zone où la perfusion est marginale. La présente investigation tente d'évaluer les propriétés protectrices de l'isoflurane dans ces circonstances. L'occlusion de l'artère cérébrale moyenne (MCAO) a été faite chez des rats Sprague-Dawley anesthésiés avec des concentrations de 1,2 MAC soit de l'halothane, soit de l'isoflurane. À la fin de la quatrième heure de MCAO, le cerveau fut extrait, sectionné et incubé avec un colorant histochimique de 2-3-triphenyltetrazolium (TTC). Le système d'analyse de l'image a été utilisé afin de mesurer la région de la réduction ou de l'absence de la coloration TTC dans quatre plans de section rayonnant à partir de l'artère cérébrale moyenne. Il n'y avait aucune différence entre les animaux anesthésiés à l'halothane et l'isoflurane quant à la région du cerveau qui a démontré une évidence de dysfonction histochimique. On conclut que l'isoflurane n'amène pas de protection (comparativement aux animaux contrôles anesthésiés à l'halothane) quand administré à des concentrations de 1,2 MAC lors d'une ischémie cérébrale focale (incomplète) de quatre heures chez les rats.

Key words

ANAESTHETICS, VOLATILE: halothane, isoflurane;
BRAIN: blood flow, protection, ischaemia.

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The investigation was carried out in the Department of Anesthesiology of the VA Medical Center, San Diego, California, and was supported by the Department of Veterans Affairs.

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Accepted for publication 14 September, 1990.

There is a risk of focal cerebral ischaemia during a variety of procedures performed under anaesthesia. As a result, there has been considerable interest in identifying anaesthetic agents that have the potential to protect the brain during cerebral ischaemia. Barbiturate anaesthesia can produce substantial suppression of cerebral metabolic rate

and has been shown to provide cerebral protection.^{1,2} However, barbiturate administration is associated with cardiovascular depression and a prolonged time to wake-up. Isoflurane is a volatile inhalational agent which, in clinically relevant concentrations, produces a degree of cerebral metabolic depression equivalent to that attainable with barbiturates.³ However, the anaesthetic effects of isoflurane are readily reversed and the time to wake-up is not prolonged. There have been numerous investigations of the cerebral protective potential of isoflurane. However, the results have been inconsistent. In spite of one provocative retrospective study that indicated that, in man, the CBF threshold for the appearance of the EEG signs of ischaemia is lower during anaesthesia with isoflurane than with other inhaled agents,⁴ the bulk of the experimental data derived from prospective investigations that have examined neurological and/or histological endpoints do not confirm the protective effect of isoflurane.⁵⁻⁸ The present study was undertaken in an attempt to provide additional laboratory confirmation of the protective potential of isoflurane. It compares the extent of histochemical dysfunction (as assessed with 2,3,5-triphenyltetrazolium chloride⁹⁻¹³ (TTC) occurring after four hours of focal cerebral ischaemia during anaesthesia with isoflurane or halothane. Halothane was selected as the "control" anaesthetic because it causes less depression of cerebral metabolic rate (CMRO₂) than equi-MAC concentrations of isoflurane, and is not commonly viewed as a cerebral protectant. The model employed (middle cerebral artery occlusion (MCAO) in the rat) is one of incomplete cerebral ischaemia. It was chosen because it is probable that it is in circumstances wherein there is a zone of marginal cerebral perfusion in which the failure of energy supply and membrane function is gradual (cf. the rapid, complete failure of energy supply associated with complete cerebral ischaemia, eg., cardiac arrest) that the protective effects related to metabolic suppression or favourable CBF redistribution are most likely to be manifest.

Methods

The protocol was reviewed and approved by the Animal Use Subcommittee of the VA Medical Center. Twenty-six male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 350-450 g were fasted overnight and were allowed unrestricted access to water. On the day of study, the animals were assigned alternately to either the isoflurane ($n = 13$) or halothane ($n = 13$) anaesthetic groups. The animals were anaesthetized in a plexiglass induction chamber with either four per cent isoflurane or three per cent halothane in oxygen. Following induction of anaesthesia, the trachea was intubated and the lungs were mechanically ventilated with 3 cm of added PEEP.

The tidal volume and rate remained constant and the inspired carbon dioxide concentration was adjusted to maintain normocarbica (PaCO₂ 35-40 mmHg). A single dose of pancuronium, 0.2 mg, was administered IV to facilitate surgical preparation. Anaesthesia was maintained with 1.2 MAC inspired concentrations of either isoflurane (1.9 per cent) or halothane (1.1 per cent) in 40 per cent oxygen/balance nitrogen. (MAC values (% $\pm$ SD) for isoflurane and halothane in Sprague-Dawley rats, as previously determined in our laboratories, are 1.58 ± 0.15 and 0.95 ± 0.11 respectively.¹⁵) Vaporizer output was confirmed prior to the start of each study with a mass spectrometer (Ohmeda 6000, multi-gas monitor, Ohmeda, Atlanta, GA). Rectal temperature was servo-controlled to 37° C with a heating pad and a heat lamp. One femoral artery and one femoral vein were cannulated with PE (polyethylene) 50 tubing. Wound margins were infiltrated with 0.25 per cent bupivacaine. The femoral artery was used for continuous measurement of mean arterial blood pressure (MAP), and for blood sampling for determination of arterial blood gases, plasma glucose concentration and haematocrit. The femoral vein was used for maintenance intravenous fluid administration (normal saline, 4 ml $\cdot$ kg⁻¹ $\cdot$ h⁻¹). MAP was maintained between 90-110 mmHg. If MAP was less than 90 mmHg, a transfusion of 3-5 ml of whole donor blood was administered. If transfusion did not raise MAP to 90 mmHg phenylephrine was infused IV. Any animal with a MAP greater than 110 mmHg at any time during the study period was excluded. Increments of sodium bicarbonate of 0.25 mEq were administered IV as necessary to maintain arterial pH greater than 7.30.

Following the vascular cannulations, a left subtemporal craniectomy was performed. The dura overlying the left middle cerebral artery (MCA) was reflected and the latter was occluded using microbipolar electrocautery under constant saline irrigation.¹⁶ The artery was coagulated between a point proximal to the olfactory tract and a second point at the intersection of the MCA with the inferior cerebral vein. Care was taken to avoid injury to the latter structure. The cauterized segment included the origin of the lenticulostriate branch. Following middle cerebral artery occlusion (MCAO) the head wound was closed with 4-0 Vicryl suture.

Anaesthesia was maintained with the original equi-MAC concentrations of isoflurane or halothane for four hours following MCAO. Physiologic data (MAP, heart rate, arterial blood gases, haematocrit) were recorded every thirty minutes. Measurements made prior to and during MCAO were averaged to obtain representative values for the two periods. Plasma glucose determinations were performed prior to MCAO and four hours later prior to sacrifice. Changes in the rate of infusion of phenyl-

FIGURE Dorsal view of the rat brain showing the location of the three coronal planes of section 3, 5 and 7 mm posterior to the frontal poles, and the relation of those planes to the approximate distribution of the middle cerebral artery.

ephrine were noted as they occurred. The time during which phenylephrine was employed, and the average rate at which it was infused when required were calculated.

At the end of the four-hour period of MCAO, the animals were sacrificed by decapitation and the brains were removed rapidly. Each brain was sectioned coronally along planes 3, 5 and 7 mm posterior the frontal pole (Figure). These sections, which span the distribution of the MCA, correspond approximately to Figures 17, 26, and 33 respectively in the atlas of the rat brain of Pellegrino *et al.*¹⁷ The four blocks of brain tissue that resulted were immersed promptly in a solution of two per cent TTC (A. Daigger & Co, Wheeling, IL) in saline and were incubated at 37° C for 30 minutes. Immediately after incubation, photographs of the brain surfaces anterior and posterior to each of the three coronal planes of section were made using Ektachrome tungsten balanced ASA 60 colour slide film (Eastman Kodak, Rochester, NY). The photographs were subsequently analyzed using a Drexel/DUMAS image processing system (Drexel University Image Processing Center, Philadelphia, PA) to determine the area of brain with reduced or absent TTC staining. The portion of the hemisphere ipsilateral to MCAO in which reduced or absent staining was evident

was expressed as a percentage of the total cross-sectional area of that hemisphere. In addition, in the same photographs, the cortex was analyzed separately and the portion that stained abnormally was expressed as a percentage of the total cross-sectional area of the entire ipsilateral cortex. The brain surfaces anterior and posterior to each coronal plane of section (3, 5, and 7 mm posterior to the frontal pole) were analyzed. The data for the anterior and posterior surfaces of each plane were averaged to yield a single value for each region (entire hemisphere, and cortex alone) in each plane. Two observers blinded to the experimental course performed the image analysis independently. The measurements made by the two observers were averaged to obtain the reported values.

Between group comparisons of the physiological data and of the percentage area of brain with abnormal TTC staining were performed using *t* tests for unpaired data. The data for the per cent area of abnormal TTC staining were analyzed both with and without an arcsine transformation. The latter was performed because the use of percentages can result in a non-normal distribution when the data points are clustered near one boundary of the available range. Within group comparisons of plasma glucose levels were performed using paired *t*-tests. A *P* value of less than 0.05 was considered significant.

Results

The weights (halothane group: 390 ± 24 g; isoflurane group: 401 ± 34) did not differ. The remaining physiological data are presented in Table I. There were no significant differences between groups for MAP, heart rate, PaCO_2 , pH, haematocrit, and plasma glucose concentration prior to MCAO. Prior to MCAO, PaO_2 in the isoflurane group (143 ± 32 mmHg) was significantly higher than in the halothane group (115 ± 23 mmHg). During MCAO heart rate and plasma glucose concentration were significantly lower in the halothane group. Plasma glucose was significantly increased in the isoflurane group four hours after MCAO but was unchanged in the halothane group.

There were no significant differences in terms of the time of infusion or the average rate of infusion of phenylephrine (Table I).

The data for the areas of brain with abnormal TTC staining are presented in Table II. In the three coronal planes examined, there were no significant differences (with or without arcsine transformation of the data) nor were any apparent trends for either the entire hemisphere or for cortex alone.

Discussion

The substantial cerebral metabolic suppression that occurs with clinical concentrations of isoflurane³ leads to

TABLE 1 Physiological data (mean $\pm$ SD) prior to (pre-MCAO) and during MCAO

	Pre-MCAO		MCAO	
	Halothane	Isoflurane	Halothane	Isoflurane
MAP* (mmHg)	94 $\pm$ 7	100 $\pm$ 3	95 $\pm$ 3	95 $\pm$ 4
Heart rate (bpm)	315 $\pm$ 44	344 $\pm$ 28	310 $\pm$ 25	345 $\pm$ 26†
PaCO ₂ (mmHg)	37 $\pm$ 2.3	36 $\pm$ 2.5	38 $\pm$ 2	37 $\pm$ 1
PaO ₂ (mmHg)	115 $\pm$ 23	143 $\pm$ 32†	141 $\pm$ 26	144 $\pm$ 26
pH	7.39 $\pm$ 0.03	7.38 $\pm$ 0.04	7.36 $\pm$ 0.02	7.35 $\pm$ 0.02
Haematocrit (%)	44 $\pm$ 3	46 $\pm$ 3	43 $\pm$ 1	42 $\pm$ 3
Plasma glucose (mg $\cdot$ dl ⁻¹)	96 $\pm$ 35	120 $\pm$ 29	117 $\pm$ 27	181 $\pm$ 74‡
Phenylephrine (μ g $\cdot$ kg ⁻¹ $\cdot$ min ⁻¹)			1.4 $\pm$ 0.7	2.3 $\pm$ 2.1
Duration of phenyl infusion (min)			160 $\pm$ 94	91 $\pm$ 92

*MAP indicates mean arterial pressure.

† $P < 0.05$ halothane vs isoflurane for pre-MCAO or MCAO data.

‡ $P < 0.05$ pre-MCAO vs MCAO.

the reasonable supposition that it might have cerebral protective properties in the setting of focal (incomplete) cerebral ischaemia. Only one relevant investigation has been performed in man and it provided support for this notion. Michenfelder *et al.*⁴ analyzed the EEG changes that occurred in patients undergoing carotid endarterectomy performed during various anaesthetic regimens. They observed that, during the period of internal carotid artery occlusion, the "critical" cerebral blood flow (the CBF above which EEG changes suggestive of cerebral ischaemia were not observed in the majority of patients) was lower during anaesthesia with isoflurane than with halothane or enflurane. However, that study, as acknowledged by the authors, was limited by the fact that it was retrospective and because the halothane and isoflurane groups were non-concurrent.

The limitations of the single human investigation mentioned above have resulted in a considerable interest in establishing definitive laboratory confirmation of the protective effect of isoflurane. However, the bulk of the relevant animal investigations, including the present study, has not provided support for a protective effect of isoflurane. Nehls *et al.* performed six hours of middle cerebral artery occlusion in baboons anaesthetized with isoflurane, barbiturate or fentanyl/N₂O.⁶ They observed a better neurological and histological outcome in the barbiturate group and accordingly could not confirm the protective effect of isoflurane. That study, however, was criticized because of differences between the groups in blood pressure and pressor doses. Milde *et al.* examined neurological and neuropathological outcome in monkeys that underwent five hours of MCAO during anaesthesia with isoflurane or barbiturate.¹⁸ They found no difference

between groups. That study, however, is also subject to criticism in that there was no control group to confirm the sensitivity of the model. Warner *et al.*⁵ compared N₂O sedated and isoflurane anaesthetized rats and found no difference in neuropathological outcome after ten minutes of forebrain ischaemia (bilateral carotid artery occlusion with simultaneous hypotension). Gelb *et al.*⁸ induced hypotension (MAP $\approx$ 50 mmHg) in monkeys during a 45-minute period of MCAO. The induced hypotension was accomplished with either sodium nitroprusside superimposed on an anaesthetic of 0.75 per cent halothane or with isoflurane alone (average end-tidal isoflurane concentration ($\pm$ SD) during hypotension: 1.9 $\pm$ 0.7 per cent). They found no differences in neurological or histological outcome. Baughman *et al.*⁷ compared the effects of isoflurane, halothane and N₂O on outcome in rats after incomplete cerebral ischaemia (unilateral carotid artery occlusion with haemorrhagic hypotension) of moderate and severe degrees. They reported that neurological and histological outcome and mortality were worse among animals that received sedation with 70 per cent N₂O than among those that received anaesthesia (1.0 MAC) with either halothane or isoflurane. However, there were no significant differences between animals anaesthetized with halothane or isoflurane.

The present study used the histochemical stain 2-3-triphenyltetrazolium as its endpoint. TTC is a colourless compound that is converted to an intense red stain (formazan) in the presence of functioning mitochondrial oxidative enzyme systems. The failure of brain tissue to convert TTC to its coloured derivative has been reported to correlate well with histopathological outcome.⁹⁻¹³ However, there is some controversy as to whether a failure

TABLE II The area (mean $\pm$ SE) of reduced or absent TTC staining in the hemisphere (HEM) ipsilateral to MCAO (expressed as a per cent of total area of the hemisphere) and in the ipsilateral neocortex (CTX) (expressed as per cent of total ipsilateral cortical area) in coronal brain sections 3, 5, and 7 mm posterior to the frontal poles

Section/region	3-CTX	3-HEM	5-CTX	5-HEM	7-CTX	7-HEM
Isoflurane	7.8 $\pm$ 1.3	9.2 $\pm$ 2.3	7.9 $\pm$ 1.5	12.2 $\pm$ 1.8	3.4 $\pm$ 2.0	4.2 $\pm$ 2.0
Halothane	6.0 $\pm$ 1.4	7.8 $\pm$ 2.2	8.2 $\pm$ 1.3	14.2 $\pm$ 1.6	1.6 $\pm$ 0.8	5.2 $\pm$ 2.0

to stain with TTC invariably represents inevitable infarction, and there are data to indicate that at least some component of the histochemical dysfunction revealed by TTC may be reversible.¹⁴ Nonetheless, a deficiency of TTC staining indicates metabolic compromise and probably represents a reasonable basis for comparing the well being of brain tissue following a period of ischaemia. It was anticipated that the beneficial effects of isoflurane, if any, would be most likely to be manifest in the penumbral zone at the periphery of the ischaemic territory. It is in these regions of marginal perfusion (as opposed to densely ischaemic core areas with severe neuronal compromise) that a protective effect could favourably tip the balance between viability and imminent demise. Accordingly, it was anticipated that a protective effect would result in a reduction of the diameter the area of abnormal TTC staining. However, the analysis revealed no difference in the areas of reduced or abnormal TTC staining between animals anaesthetized with halothane and isoflurane.

In one phase of the present analysis, the neocortex was examined separately. This was done because it has been demonstrated that, while isoflurane in general causes a greater suppression of cerebral metabolic rate (CMR) than halothane, the differences are greater in the cortex than in sub-cortical structures.¹⁹ It might therefore be anticipated that the cerebral protective effects of isoflurane, if any, would be most obvious in cortex. It seemed feasible that, with a less selective analysis, cerebral protective effects might be obscured by the influences of damage to regions that were not subject to the protective effects because of differences in their CMR response to isoflurane. Nonetheless, selective protection of the cortex was not apparent.

There were differences in physiological variables between the two groups. During MCAO heart rate was significantly lower in the halothane group. This finding is consistent with properties described in standard references.²⁰ As well, PaO₂ was significantly less in the halothane group (115 $\pm$ 23 mmHg) than in the isoflurane group (143 $\pm$ 32) prior to MCAO but this difference was not apparent during MCAO. It is unlikely that these minor physiological differences significantly influenced the TTC results.

Plasma glucose concentrations also differed. The isoflurane group had a significantly greater glucose level after four hours of MCAO. This finding is consistent with other studies that have shown that isoflurane anaesthesia is associated with significantly higher plasma glucose levels than other inhaled agents in both man and animals.^{21,22} This phenomenon may be the result of a decreased insulin response to glucose.²³ Numerous investigations have confirmed that hyperglycaemia increases neurological injury during CNS ischaemia.^{24–27} Therefore, it is possible that a protective effect of isoflurane may have been negated in the present study by the higher glucose levels in the isoflurane anaesthetized animals.

The present study evaluated the effects of a four-hour period of MCAO. It is possible that the protective potential of isoflurane will only be apparent in circumstances of near-threshold injury, and that the insult in the present experiment was too severe. The four-hour ischaemic time was chosen because of our preliminary observations that, in this species, the threshold for reliable development of a TTC deficit is approximately three hours. While the location of the salvageable periphery might well shift with increasing duration of occlusion, it seems probable that there would still be a marginally perfused region at the periphery of the ischaemic territory that would constitute a threshold injury state and that, in the event of some protective effect, the dimension of the TTC-deficient area would be influenced.

Our previous experience with this model has confirmed its capacity to identify a beneficial intervention. Measurement of the area of TTC deficiency has been employed in two previous investigations in our laboratories.^{14,28} In those studies, TTC measurements were made either three or five hours after vessel occlusion and in both, therapeutic interventions (induced hypertension²⁸ and re-establishment of vessel patency¹⁴) were shown to diminish the size of the TTC lesion. We are nonetheless aware that the variability of TTC lesion size with this model is substantial and presents a limitation. For instance, the power of this model to confirm a 50 per cent reduction in lesion size at the centre of the ischaemic area is approximately 0.70.²⁹ However, the absence of even apparent trends toward a reduction of the area of injury in

isoflurane anaesthetized animals (Table II) makes it unlikely that a substantial protective effect by isoflurane has been overlooked.

The concentration of isoflurane employed, 1.2 MAC, may have been a factor. It is possible that this concentration is inadequate to provide protective effects, although previously available information leads to the expectation that it should be sufficient. The retrospective study of Michenfelder *et al.* of critical CBF during carotid endarterectomy⁴ and the small prospective investigation of Casement *et al.*²⁷ indicated a protective effect for isoflurane in concentrations as low as 0.6 MAC. It has been surmised that the protective properties of isoflurane are the result of the suppression of CMR that it causes. A 1.2 MAC concentration of isoflurane is sufficient to cause a substantial suppression of CMR. In rats, the cerebral metabolic rate for glucose (CMRg) in the sensorimotor cortex is reduced to 44 per cent of awake control values by 1.0 MAC isoflurane anaesthesia,³⁰ although additional reduction of CMRg does occur at higher concentrations. In addition, it has been demonstrated that, in this concentration range, isoflurane produces significantly greater metabolic depression than does 1.0 MAC halothane. In cats, a 1.0 MAC end-tidal concentration of isoflurane caused a 45 per cent reduction of CMRO₂ (as compared with a nitrous oxide-sedated control state) while an equivalent MAC concentration of halothane caused a decrease of only 27 per cent.³¹ In rats, during 1.0 MAC isoflurane anaesthesia, CMRg in the neocortex was 36 per cent less than that observed during 1.0 MAC halothane anaesthesia.¹⁹ Accordingly, it seems likely that the 1.2 MAC concentration employed in the present study should have been sufficient to provide any protective benefit related to CMR suppression.

Phenylephrine was used to support blood pressure to equivalent levels in the two anaesthetic groups. There were no statistical differences between the infusion rates or total duration of infusion of phenylephrine; however, there was substantial variation between animals. Nonetheless, the phenylephrine is unlikely to have influenced the results as it has been previously demonstrated in Sprague-Dawley rats during MCAO that phenylephrine does not result in cerebral vasoconstriction in either normal or ischaemic brain.³²

In summary, the present study compared the effects of halothane and isoflurane on brain injury as measured by abnormal TTC staining after four hours of focal cerebral ischaemia in the rat. There were no significant differences in the size of the area of abnormal TTC staining. The present results do not provide support for the cerebral protective properties that have been attributed to isoflurane.

Acknowledgements

The authors are grateful for the technical assistance of Robin Giamela, M.S. and Joanne Steinauer. This work was supported by the Veterans Administration and The Canadian Heart and Stroke Foundation.

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