Minimum alveolar concentration of halogenated volatile anaesthetics in left ventricular hypertrophy and congestive heart failure in rats

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Background. Although many physiological and pathological conditions affect minimal alveolar concentration (MAC), there are no reliable data on the MAC for halogenated anaesthetics during left ventricular hypertrophy (L VH) and congestive heart failure (CHF). The aim of this experimental study was to determine the MAC values of halothane, isoflurane, and sevoflurane in rats, at early and later stages of cardiomyopathic hypertrophy.

Methods. L VH was induced by ascending aortic stenosis in 3–4-week-old rats. L VH and CHF in each animal were assessed weekly by echocardiography. MAC of halothane, isoflurane, and sevoflurane was determined using the tail-clamp technique in spontaneously breathing rats from each group. Response vs no-response data were analysed using logistic regression analysis. Data are medians (95% confidence interval).

Results. The MAC of halothane [1.30% (1.26–1.34)], isoflurane [1.52% (1.48–1.57)], and sevoflurane [2.93% (2.78–3.07)] in rats with L VH was not different from sham-operated rats [respectively, 1.23% (1.20–1.26), 1.52% (1.47–1.56), and 2.90% (2.79–3.00)]. Conversely, the MAC of halothane [1.44 (1.39–1.50)] and isoflurane [1.74 (1.69–1.78)], but not sevoflurane [2.99 (2.93–3.06)], was significantly increased in rats with CHF.

Conclusions. MAC values for halothane, isoflurane, and sevoflurane were unchanged in rats with pressure-induced overload L VH. Conversely, the MAC for halothane and isoflurane, but not sevoflurane, was significantly increased in rats with CHF.

Br J Anaesth 2007; 99: 787–93

Keywords: anaesthetics volatile, halothane; anaesthetics volatile isoflurane; anaesthetics volatile, sevoflurane; heart, myocardial function; potency, anaesthetic, MAC

Accepted for publication: July 17, 2007
genetically induced model of cardiomyopathy. Indeed, the mechanism underlying the MAC reduction between healthy and cardiomyopathic hamsters in this study could have been due to either genetic differences between strains or consequences of cardiomyopathy per se.

Therefore, the aim of this experimental study was to determine MAC values of halothane, isoflurane, and sevoflurane in rats with pressure-overload cardiomyopathic hypertrophy. This experimental model offers the opportunity to investigate rats of the same strain, at the early stage of LVH and at the later stage of congestive heart failure (CHF).

Methods

Animals
Care of the animals conformed to the recommendations of the Helsinki Declaration, and the study was performed in accordance with the regulations laid down by the French Ministry of Agriculture. These experiments were conducted in an authorized laboratory under the supervision of an authorized researcher (B.R.). Male Wistar rats were obtained at the age of 3 weeks from Charles River Laboratories (St Germain sur l’Arbresle, France). Rats were given food and water ad libitum, and a 12 h light–dark cycle was provided.

Induction of left ventricular hypertrophy
LVH was induced by ascending aortic stenosis, as previously described. Briefly, a stainless-steel clip of 0.6 mm internal diameter was surgically placed via a left thoracic incision onto the ascending aorta in 3–4-week-old rats, in order to induce a pressure-overload hypertrophic cardiomyopathy. Sham-operated animals were subjected to the same procedure except the aortic stenosis. The first stage of this rat experimental model is characterized by the appearance of LVH between the age of 12 and 14 weeks. The final stage is characterized by the occurrence of CHF, which is generally observed at an age of 23 weeks.

Assessment of LVH was monitored weekly by echocardiography from the age of 10 weeks, until the diagnosis of LVH had been established in each animal. Nineteen weeks after aortic stenosis, the animals were observed daily to allow the detection of tachypnoea, which is an early clinical sign of cardiac dysfunction. Thereafter, echocardiography was repeated weekly until the diagnosis of CHF had been established in each animal. During echocardiographic evaluation, animals were lightly anaesthetized (1.0% isoflurane, inspired fraction in oxygen, 3 litre min⁻¹), and the left part of the anterior chest shaved. Transthoracic M-mode and two-dimensional echocardiography was performed with an Acuson 128XP imaging system with a 12 MHz broadband transducer (Acuson Corp., Mountain View, CA, USA). The following echocardiographic variables were measured: internal end-systolic (ESLVD) and end-diastolic (EDLVD) left ventricular diameters, interventricular septum thickness (IVS), posterior wall thickness (PWT), and fractional shortening. Left ventricular mass (LVM) was estimated using the following equation, where 1.04 indicates the specific density of the myocardium:

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\text{LVM} = 1.04 \cdot \left[ (\text{IVS} + \text{EDLVD} + \text{PWT})^3 - \text{EDLVD}^3 \right] 
\]

Each set of measurements was obtained from the same cardiac cycle. At least three sets of measurements were obtained from three different cardiac cycles. Normalized LVM was determined by dividing the LVM by the mean value of LVM in the control group and expressed in percentage. LVH was defined by a >35% increase in LVM. CHF was defined by a >25% decrease in fractional LV shortening, associated with clinical signs of heart failure such as tachypnoea, pleural effusion, and ascites.

After MAC determination, 15 sham-operated rats, 15 rats with LVH, and 15 with CHF were killed for assessment of cardiac hypertrophy. Heart weight to body weight and left ventricular weight to body weight ratios were calculated. The degree of cardiac hypertrophy was determined by dividing the heart weight to body weight value of each rat with CHF or LVH by the mean heart weight to body weight value in sham-operated rats, as previously reported. Thereafter, the degree of cardiac hypertrophy was compared with the LVM previously estimated in the animal echocardiographically.

Experimental protocol
Four groups of rats were used for MAC determination. The first group contained rats with ascending aortic stenosis investigated at the stage of LVH, as assessed by echocardiography (LVH group). The second group contained rats with ascending aortic stenosis investigated at the stage of CHF, as assessed by echocardiography (CHF group). The third group contained the sham-operated rats (Sham group). Finally, a fourth group of rats without any surgical procedure was also investigated as controls (control group). The Sham and control groups were investigated contemporaneously and at the same age as the LVH group.

MAC determination was performed for halothane, isoflurane, and sevoflurane in each group of rats. Nine to 12 rats were used simultaneously in each experiment with one halogenated anaesthetic, and at least three experiments with the same anaesthetic were required to complete MAC determination in one group. Animals of one group were eventually re-studied with different halogenated anaesthetic, but a minimal interval of 2 days was required between the two experiments involving the same animals.
Most of the rats investigated at the stage of LVH (LVH group) were also investigated later at the stage of CHF (CHF group). Finally, to verify that the isoflurane anaesthesia used to facilitate weekly echocardiographic measurements did not alter MAC, we also determined MAC of isoflurane in an additional group of rats before and 7 weeks later after weekly 1.0% isoflurane anaesthesia.

MAC was determined using the tail-clamp technique, as previously described. Animals were tested at the same time of the day, to minimize variations in anaesthetic requirements induced by circadian rhythm. Spontaneously breathing rats were exposed to halothane, isoflurane, or sevoflurane in individual chambers (24×22×13 cm) hermetically closed with a thin plastic sheet. The volatile agent was vaporized with a calibrated vaporizer (Fluotec 4, Isotec 4 and Sevotec 5; Ohmeda, Steeton, UK) in 100% oxygen as the carrier gas, with fresh gas flow of 2 litre min⁻¹ in each chamber. Concentrations of the halogenated anaesthetic in each chamber were measured with a calibrated infrared analyser (Datex-Ohmeda 5250 RGM, Limonest, France). The infrared analyser was calibrated daily according to the manufacturer’s guidelines using anaesthetic mixtures of known concentration.

Concentrations of the halogenated anaesthetic in each chamber were measured with a calibrated infrared analyser (Datex-Ohmeda 5250 RGM, Limonest, France). The infrared analyser was calibrated daily according to the manufacturer’s guidelines using anaesthetic mixtures of known concentration. Chambers were placed on an electric warming blanket and their temperature was continuously monitored (Homeothermic Blanket System, Harvard Apparatus, Inc., South Natick, MA, USA). In addition, the body temperature of one animal in each experiment was continuously monitored using a rectal probe (Small Flexible Vinyl Rectal/Esophageal Probe, Harvard Apparatus). When the rectal temperature of this rat dropped by 1.0°C, the chambers were then re-warmed using the warming blanket and a heating lamp until the temperature was restored to 37.0°C.

Before applying the first-test stimuli, rats were exposed for 1 h to a constant anaesthetic concentration of almost 80% of the halothane, isoflurane, and sevoflurane MAC values previously determined at 37.0°C in adult Wistar rats, which were, respectively, 1.11%, 1.38%, and 2.50%. The 1 h exposure time was chosen to achieve inspiratory (FI) to alveolar (FA) fraction ratios close to 1.0 and to maintain the total anaesthetic exposure time to <6 h.

A laparoscopic surgery clamp was applied for 60 s to the first ratchet position on the mid portion of the tail of the rat without wiggling the clamp, as previously described. The clamp was applied through a small hole in the plastic sheet so as not to modify the anaesthetic concentration in the experimental chamber. An animal was considered to have moved if it made a ‘gross purposeful muscular movement’ usually of the hind limb or the head, as opposed to simply increasing its rate of depth of respiration. The anaesthetic concentration was increased by steps of 0.1% (halothane and isoflurane) to 0.2% (sevoflurane) and the testing sequence was repeated after 30 min of exposure to each concentration, indicating that the steady-state FI/FA ratios close to 1.0 could be reasonably achieved. No experiment required exposure to more than eight consecutive increased anaesthetic concentrations; therefore, the total anaesthetic exposure was <6 h. However, MAC determination is not affected by duration of anaesthetic exposure. At the end of the procedure, anaesthetic administration was stopped and rats awoke while breathing 100% oxygen.

### Statistical analysis

The original MAC concept of Eger and colleagues used a ‘bracketing approach’ in humans and animals. In animal studies, it is possible to apply the tail-clamp stimuli on multiple occasions. Thus, an appropriate mathematical technique to quantify the relationship between MAC and response is no response data is logistic regression analysis. Such analyses show the probability of binary outcome (i.e. yes or no response) as a linear function of the exponential part of logit of the logistic function. This model may be applied to MAC determination. The equation enabling calculation of MAC from logistic regression has been previously described. This produces values for MAC comparable with those produced with the bracketing technique and enables an extrapolation of the probability of response to any given anaesthetic concentration within the curve. For each group of rats and for each volatile anaesthetic, median MAC value using logistic regression (NCSS 2001 software, Statistical Solutions Ltd, Cork, Ireland) and the 95% confidence interval were calculated. All P-values were two-tailed, and a P-value <0.05 was considered significant.

### Results

During determination of MAC, two rats from the LVH group died from respiratory distress due to excessive exposure to isoflurane for one animal and sevoflurane for the other. Otherwise, all animals recovered without obvious untoward effect. No further deaths occurred during the 2 days after experimentation.

Weekly echocardiographic assessment revealed that LVH was present generally after the age of 14 weeks. Similarly, weekly echocardiographic assessment from the age of 22 weeks revealed that CHF was present generally after the age of 34 weeks.

Body weights were not significantly different between LVH and sham-operated rats (Table 1). Heart weight and left ventricular weight in LVH and CHF rats were significantly greater than those of sham-operated and control rats. Consequently, the heart weight to body weight and left ventricular weight to body weight ratios were significantly greater in LVH and CHF rats, indicating cardiac hypertrophy.

Figure 1 presents the comparison between LVM calculated from echocardiographic measurement and left ventricular weight measured after death, for sham-operated LVH.
In all groups, there was a good correlation between these two variables. The MAC of isoflurane in control rats before and 7 weeks later, after weekly repeated 1.0% isoflurane anaesthesia, was not significantly different [respectively, 1.47% (1.42–1.51) vs 1.46% (1.42–1.50), NS], indicating that repeated echographic assessments under isoflurane anaesthesia did not interfere with MAC determination.

MAC values are presented in Table 2 and Figure 2. MAC values were not significantly modified in the LVH group, whatever halogenated anaesthetic was used. For halothane and isoflurane, MAC in the CHF group was significantly increased when compared with the control, Sham, and LVH groups. Conversely, for sevoflurane, MAC in the CHF group was not significantly modified when compared with the control, Sham, and LVH groups.

**Discussion**

In this study, we have shown that (i) the MAC of halothane, isoflurane, and sevoflurane in rats with LVH was not significantly modified and (ii) the MAC of halothane and isoflurane, but not sevoflurane, was significantly increased in rats with CHF when compared with healthy rats and rats with LVH.

To the best of our knowledge, our study is the first to demonstrate a change in volatile anaesthetic MAC during CHF due to long-term pressure-overload. We have previously reported a decrease in MAC for halothane, isoflurane, sevoflurane, and desflurane in hamsters with cardiac hypertrophy, but this model involved a genetically induced pathway, and therefore, the mechanism underlying the MAC reduction in this study could have been either genetic differences between strains or consequences of cardiomyopathy per se.13

Onset of LVH after ascending aortic stenosis performed in 3–4-week-old rats usually occurs around the 12–14th week, whereas transition to CHF generally occurs around the 23–27th week.14 Using the same model, Moreira and colleagues15 reported that the time elapsed from aortic stenosis until appearance of tachypnoea, which is usually the first clinical sign of CHF, is highly variable, from 29 to 37 weeks. In our study, LVH was effectively present in rats after the age of 14 weeks, similar to Miyamoto’s study.14 Conversely, CHF was noted only after the age of 34 weeks, similar to Moreira’s study.15 This delay in appearance of CHF when compared with Miyamoto and colleagues14 could be due to the difference in the internal diameter of the clip (0.60 mm in our study and in Moreira’s study vs 0.58 mm in Miyamoto’s study) or minor technical difference during the production of the aortic stenosis. On the other hand, since anaesthesia is mandatory to perform echographic assessment correctly in rats, the anaesthetic agent chosen could also explain our results. Indeed, Iltis and colleagues27 have recently showed that rats under isoflurane anaesthesia had higher ejection fraction than those under pentobarbital anaesthesia. Since we used isoflurane for echographic assessment, whereas Miyamoto and colleagues14 used pentobarbital,
we could have slightly overestimated LV function during each echography, and consequently could have delayed the diagnosis of the onset of CHF.

The MAC values for halothane, isoflurane, and sevoflurane that we observed in healthy rats were slightly greater than those previously reported in adult Wistar rats, which were, respectively, 1.11%, 1.38%, and 2.50%. Variations in MAC determination may result from subtle differences in the technique of measurement. For example, reported MAC values for halothane in adult Wistar rats range from 0.8 (0.1)% to 1.3 (0.1)%. Nevertheless, whatever the anaesthetic investigated, our MAC values in control and sham-operated groups were very close, indicating reliability of our measurement method.

Since we observed an increase in MAC of halothane and isoflurane in CHF rats, we considered whether this increase could have been related to weekly anaesthesia performed for echographic assessment, for example, through a tolerance mechanism. Eger and colleagues had previously reported that MAC deviation of halothane was only 0.07 (0.05)% between two MAC determinations separated by at least 2 weeks. Indeed, to the best of our knowledge, the effect of weekly repeated administration of volatile halogenated anaesthetics on their MAC value has not yet been investigated. Our experiment clearly showed that the MAC of isoflurane in rats remained unchanged after a 7 week period with weekly repeated isoflurane anaesthesia.

The classical way to approach MAC variation during cardiac disease would involve a haemodynamic mechanism, since hypotension has been previously reported to reduce anaesthetic requirements. Conversely, the explanation for the increase in MAC that we observed during CHF should involve other mechanisms, such as a neurotransmitter pathway. Several hypotheses can be proposed. First, i.v. administration of catecholamines such as ephedrine, but not epinephrine or norepinephrine, has been shown to increase the MAC of halogenated anaesthetics. Ephedrine produces central nervous system simulation through central release of norepinephrine, and an increase in sympathetic activity occurs during CHF. Another pathway which could be involved in the change in MAC during CHF is the opioid system. Indeed, circulating concentrations of beta-endorphin have been reported to be increased in canine and human models of CHF. However, the anaesthetic potency of halogenated anaesthetics is enhanced by the endogenous opioid system, which would imply a decrease in their MAC values. Finally, our experimental model involves an ascending aortic stenosis, and since partial aortic obstruction has been shown to improve cerebral perfusion, a relationship between such an increase in cerebral blood flow and the increase in MAC values during CHF could also be hypothesized. Nevertheless, since we did not study the possible mechanisms involved in the increase in MAC during CHF,
the suppositions above (which would apply only to halothane and isoflurane in our study) will require further experimental evaluation.

We observed a significant increase in MAC in the CHF group for halothane and isoflurane, but not for sevoflurane, which is the less soluble agent. An explanation for these differences would involve their diffusion properties, as previously reported. Indeed, determination of MAC assumes that there is no barrier to the diffusion of the volatile anaesthetic from alveolar gas into arterial blood, and hence to the central nervous system (CNS). However, CHF could have induced a barrier to such diffusion for the more soluble agents, that is, halothane and isoflurane, and therefore increased the inspired concentration required to achieve a given concentration in the CNS. In our experiments, the 30 min exposure time at each concentration was chosen to favour steady-state FIF/FA. Nevertheless, only measurements of arterial partial pressures of halogenated anaesthetics relative to their inspired concentrations would enable verification of steady state and measurement of the true arterial partial pressure needed to produce immobility. Finally, whereas volatile halogenated anaesthetics between 0.5 and 1.5 MAC increase cerebral blood flow velocity through an intrinsic dose-dependent vasodilatory action, Matta and colleagues have reported that this effect was less pronounced for sevoflurane than for halothane and isoflurane. The relationship between this weaker cerebral vasodilatory effect of sevoflurane and the absence of an increase in MAC values during CHF for sevoflurane, when compared with halothane and isoflurane, requires further investigation.

Some remarks must be included to address the limitations of our study. First, MAC was classically considered as unaffected by the type of stimulation, providing that a supra-maximal stimulus was applied. However, movements resulting from noxious stimuli may be variable in quality and/or intensity, and appreciation of ‘gross purposeful muscular movement’ may be subjective. Finally, for peri-MAC and/or intensity, and appreciation of ‘gross purposeful muscular movement’ may be subjective.6

In conclusion, the MAC of halothane and isoflurane, but not sevoflurane, is significantly increased in rats with CHF compared with healthy rats and rats with LVH. These results are important in the evaluation of the effects of halogenated anaesthetics in experimental models of cardiac disease. Further studies are required to explain this increase in MAC during CHF.

Funding
Fondation pour la Recherche Médicale (to T.B.); Université Pierre et Marie Curie—Paris VI; departmental sources.

Acknowledgement
We thank Dr David Baker, DM, FRCA (Department of Anesthesiology and Critical Care, Hôpital Necker-Enfants Malades, Paris), for reviewing the manuscript.

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