

Halothane, Isoflurane, and Enflurane MAC in Pregnant and Nonpregnant Female and Male Mice and Rats

Richard I. Mazze, M.D.,* Susan A. Rice, Ph.D.,† Jeffrey M. Baden, M.D.‡

The MAC of halothane, isoflurane, and enflurane was determined using the tail-clamp technique in pregnant female, nonpregnant female, and male Swiss Webster mice (n = 216) and Sprague-Dawley rats (n = 112). Mean MAC values (\pm SD) for halothane, isoflurane, and enflurane in mice were $0.95 \pm 0.07\%$, $1.34 \pm 0.10\%$, and $1.95 \pm 0.16\%$, respectively; values in rats were $1.03 \pm 0.04\%$, $1.46 \pm 0.06\%$, and $2.21 \pm 0.08\%$, respectively, all significantly higher than in mice. Neither the sex of the animals nor whether female animals were pregnant influenced the results. (Key words: Anesthetics, volatile: halothane; isoflurane; enflurane. Potency, anesthetic: MAC. Pregnancy.)

THE MINIMUM ALVEOLAR CONCENTRATION (MAC) at which 50% of subjects make purposeful movements in response to a supramaximal stimulus is a widely used measure of the potency of inhalation anesthetic agents.¹⁻³ Recently, while designing reproductive studies utilizing rats and mice, we searched the literature for MAC values of halothane, isoflurane, and enflurane for pregnant mice. We could not find them for mice nor, with the exception of halothane,⁴ could we find MAC values for pregnant rats. The present study provides these data for pregnant and nonpregnant female and male mice and rats.

Methods

MICE

Groups of 12 pregnant female, 12 nonpregnant female and 12 male, 9- to 10-week-old Swiss Webster mice§ were housed, by sex, four to a cage. Mice were bedded on ground corncob¶ and fed a

standard laboratory animal diet** and tap water *ad libitum*. The animal quarters were maintained at $21 \pm 1^\circ$ C and $55 \pm 10\%$ humidity with light present from 0600 to 1900 h. On day 9 of pregnancy (day 0 was the day a copulatory plug was observed), groups of pregnant female, nonpregnant female, and male mice were placed together in a plexiglass chamber of approximately 1,000 l volume for determination of MAC. Thus, 36 mice were studied at a time. Animals were tested at the same time of day, from 1300 to 1800 h, and only one agent was used in any one study.

The volatile agent was vaporized with compressed air as the carrier gas. Supplemental oxygen was added so that ambient oxygen concentration was maintained between 21-25%. The chamber was charged with the volatile agent for 3-5 min at concentrations estimated to be well in excess of MAC at a total gas flow of $15-20$ l·min⁻¹. Following this, gas flow was reduced to $5-7$ l·min⁻¹ and the anesthetic concentration was reduced to a maintenance level estimated to be 70-80% of MAC. This concentration was maintained for at least 2 h and 30 min prior to beginning MAC determinations. Anesthetic concentrations in the chamber were determined at 1-15-min intervals, using a Varian® 1440 gas chromatograph. Trends in anesthetic concentrations were followed with an Engstrom® EMMA quartz crystal monitor. Body temperature of two animals in each group was measured continuously with a Yellow Springs Tele-Thermometer.® When average temperature dropped by 1° C, a water mattress under the floor of the chamber was turned on. It was turned off when the temperature returned to the initial values.

MAC was determined by clamping a 6-inch hemostat to the first rachet position on the mid-portion of the tail. If the animal made a purposeful movement within 1 min, as opposed to increasing its rate of depth of respiration (a frequent response), it was considered to have moved. If more than 50% of mice moved, the anesthetic concentration was increased by approximately 10%, and 20-30 min later the testing sequence was repeated. The experiment was terminated when 50% or more of the mice in each group failed to move in response to tail-clamping. If exactly 50% failed to move, the concentration was MAC. If less than 50% moved, then MAC was considered to have been exceeded, and its value was interpolated between the two closest concentrations. An average of four concentrations of each agent were tested in each test sequence. At the end of the experiment, pregnant mice were killed and the uterus was examined to verify pregnancy. The entire experiment was replicated once, thus, a total of 216 mice were used. MAC was determined for each group of 12 mice in each replicated experiment. Average replicate values are presented in the table. Mean MAC values for each anesthetic for the six determinations (\pm SD) are reported.

Since only inspired anesthetic concentration was measured, a correction was necessary to estimate alveolar concentration. In a previous study of MAC in rats, White *et al.*⁵ measured both inspired and end-tidal anesthetic concentrations of halothane and isoflurane. Two to 3 h after induction of anesthesia, the inspired-to-alveolar

* Professor of Anesthesia, SUSM, and Chief, Anesthesiology Service, PAVAMC.

† Assistant Professor of Pharmacology and Toxicology in Anesthesia, SUSM, and Pharmacologist, PAVAMC.

‡ Associate Professor of Anesthesia, SUSM, and Staff Anesthesiologist, PAVAMC.

Received from the Departments of Anesthesia, Stanford University School of Medicine (SUSM), and Palo Alto Veterans Administration Medical Center (PAVAMC), Palo Alto, California. Accepted for publication September 11, 1984. Supported by the Veterans Administration, NIH grant GM 22746, and Ohio Medical Anesthetics.

Address reprints to Dr. Mazze: Anesthesiology Service (112A), Veterans Administration Medical Center, 3801 Miranda Avenue, Palo Alto, California 94304.

§ Hla:(SW)BR; Hilltop Lab Animals, Inc., Scottsdale, Pennsylvania 15683.

¶ Bed-O'Cobs,® Anderson's Cob Division, Maumee, Ohio 43537.

** Wayne Lab-Blox,® Allied Mills, Inc., Chicago, Illinois 60951.

TABLE 1. MAC Values for Mice and Rats (%)*

	Pregnant Female	Nonpregnant Female	Male	Mean \pm SD
Mice				
Halothane	0.98	0.96	0.89	0.95 \pm 0.07 (6)†
Isoflurane	1.35	1.35	1.32	1.34 \pm 0.10 (6)
Enflurane	2.08	1.97	1.85	1.95 \pm 0.16 (6)
Rats				
Halothane	1.02‡	1.03	1.04‡	1.03 \pm 0.04 (4)
Isoflurane	1.43‡	1.52	1.44	1.46 \pm 0.06 (5)
Enflurane	2.22‡	2.17	2.25	2.21 \pm 0.08 (5)

* Average for replicate experiments.

† Number of experiments are in parentheses.

‡ Not replicated.

difference was 9–13% for halothane and 3–6% for isoflurane. Thus, we used a correction of 11% for halothane and 4% for isoflurane. We used an inspired-to-alveolar correction of 7.5% for enflurane, based on its blood-gas partition coefficient of 1.8, a value that is approximately midway between that of halothane and isoflurane.

RATS

Using the methods described above, MAC was determined for halothane, isoflurane, and enflurane in groups of 9-week-old Sprague-Dawley rats.†† Each group contained eight rats, and replicate experiments were performed only as indicated in table 1. A total of 112 rats were used.

Results

The calculated MAC values or the average MAC values of replicated experiments, are shown in table 1. Also, the mean value (\pm SD) for all groups for each agent are given. Mean body temperature in rats ranged from 36.3–36.8°C. Body temperature in mice were similar, but exact values were not recorded. The replicated experiments for each group of animals were in close agreement with each other; the overall mean difference between replicates were 5.7 \pm 1.5% for mice and 11.7 \pm 4.2% for rats. There were no consistent differences in MAC values related to the sex of the animals or to whether female animals were pregnant. Values in rats were significantly greater than in mice for each agent (Student's *t* test; *P* < 0.05).

Discussion

In searching the literature for MAC data for pregnant rodents, we found only the report of Strout and Nahrwold, who administered halothane to rats.⁴ Thus, the present study adds important information to the data base for the three most commonly used potent inhalation

anesthetics. We also noted that the methods for determining MAC in animals were described incompletely or inconsistently. White *et al.*⁵ who studied rats, refers to the methods of Eger *et al.*,⁶ who studied dogs. However, White *et al.* clamped the distal third of the tail, while Eger *et al.* stated they clamped a 10-inch hemostat about 2–4 inches from base of the tail until the ratchet caught. Eger *et al.*⁶ used 20% steps in anesthetic concentration, interpolating MAC values when they fell between two concentrations. Quasha *et al.*,³ while not mentioning animal species in their review article, state that to determine MAC, a full-length hemostat is applied close to the base of the tail and clamped to full ratchet lock; they note that 10% rather than 20% step changes in anesthetic concentration result in a more precise determination of MAC. Strout and Nahrwold⁴ refer to White *et al.*⁵ for their methods for determining MAC. None of the papers discusses whether MAC should be approached from a lower concentration, a higher concentration, or from both directions. Given this lack of specificity, we standardized our technique as described in the "Methods" section and used 10% step increases in anesthetic concentration. A uniform technique is important because in pilot studies we noted that when rats were tested within moments of each other at the same anesthetic concentration, more than 50% moved (ergo, concentration < MAC) when a clamp was applied to the base of the tail, whereas less than 50% moved (ergo, concentration > MAC) when the same stimulus was applied to the mid-portion of the tail. Depending on the steepness of the MAC dose-response curve (usually very steep), this could represent differences in the experimentally determined value of MAC, ranging from only a few percent to perhaps as much as 10–15%.

As in most of the previous determinations of MAC in rodents, inspired rather than alveolar anesthetic concentrations were measured and corrections were applied.³ We used a factor based on the experiment of White *et al.*,⁵ in rats and our MAC values for halothane and isoflurane, 1.03% and 1.46%, respectively, were similar to theirs, 1.11% and 1.38%. We used the same correction factor in mice. This may have been excessive, since mice are only one-tenth the size of rats and have less dead space. However, on average, our values in mice were only 7% lower than those of Deady *et al.*,⁷ who also measured only inspired anesthetic concentrations. They went from high to low anesthetic concentrations in 20% increments and then back to high concentrations. MAC was assumed to be the crossover point; correction factors were not applied. The close agreement of MAC values in our study and theirs suggests that the correction factors we used were appropriate. As discussed, MAC has been determined by going from a high anesthetic

†† Hla:(SD)BR; Hilltop Lab Animals, Inc., Scottsdale, Pennsylvania 15683.

concentration to a low one, from a low concentration to a high one and by averaging the results of the two techniques. Clearly, the most accurate data are obtained when alveolar concentrations are measured. If only inspired concentrations are determined, going from high to low concentrations is preferred, as this technique will result in lower inspired-alveolar concentration differences.

It is interesting that we did not find differences in MAC values between pregnant and nonpregnant female mice and pregnant and nonpregnant rats. Strout and Nahrwold⁴ studied Sprague-Dawley rats and reported that MAC in pregnant rats was 19% lower than in nonpregnant animals. Palahniuk *et al.*⁸ reported reductions in MAC of 25% for halothane, 40% for isoflurane, and 32% for methoxyflurane in pregnant compared with nonpregnant ewes. It has been postulated that decreased anesthetic requirement was related to increased progesterone levels, which occur in pregnancy. However, Strout and Nahrwold⁴ reported increased MAC values on days 10 and 21-23 of pregnancy; they note that progesterone levels are elevated on day 10 but not on day 21. Thus, there must be other explanations for the differences in our results and theirs. Until

these discrepancies are clarified, investigators and clinicians should view the issue of MAC reduction during pregnancy with some circumspection.

References

1. Merkel G, Eger EI II: A comparative study of halothane and halopropane anesthesia. Including method for determining equipotency. *ANESTHESIOLOGY* 24:346-357, 1963
2. Saidman LJ, Eger EI II: Effect of nitrous oxide and of narcotic premedication on the alveolar concentration of halothane required for anesthesia. *ANESTHESIOLOGY* 25:302-306, 1964
3. Quasha AL, Eger EI II, Tinker JH: Determination and applications of MAC. *ANESTHESIOLOGY* 53:315-334, 1980
4. Strout CD, Nahrwold ML: Halothane requirement during pregnancy and lactation in rats. *ANESTHESIOLOGY* 55:322-323, 1981
5. White PF, Johnston RR, Eger EI II: Determination of anesthetic requirement in rats. *ANESTHESIOLOGY* 40:52-57, 1974
6. Eger EI II, Saidman LJ, Brandstater B: Minimum alveolar anesthetic concentration: A standard of anesthetic potency. *ANESTHESIOLOGY* 26:756-763, 1965
7. Deady JE, Kolbin DD, Eger EI II, Heavner JE, D'Aoust B: Anesthetic potencies and the unitary theory of narcosis. *Anesth Analg* 60:380-384, 1981
8. Palahniuk RJ, Shnider SM, Eger EI II: Pregnancy decreases the requirement for inhaled anesthetic agents. *ANESTHESIOLOGY* 41:82-83, 1974