A Method for Determining Minimum Alveolar Concentration of Anesthetic in the Rat

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Minimum alveolar concentrations (MAC) for halothane and methoxyflurane were determined at two stimulus intensities in the rat. Forty-three rats were anesthetized with halothane or methoxyflurane in oxygen, and after tracheostomy were ventilated with a Harvard Rodent Respirator. Anesthetic agents were delivered by a nonbreathing circuit, with the animal's body temperature maintained at 37 ± 0.1 C. End-tidal gas was obtained by intermittently stopping the respirator at end-expiration and withdrawing 0.2–0.3 ml of gas from the tracheostomy. When three successive end-tidal samples taken over 15 minutes had the same anesthetic concentration, the rat's tail was clamped with either a bulldog artery clamp or a hemostat and the response noted. MAC was determined as the mean of the lowest alveolar concentration preventing and the highest permitting movement in response to the two stimuli. For halothane, MAC's were 0.82 ± 0.12 per cent when the bulldog clamp was used in eight rats and 1.17 ± 0.51 per cent with the hemostat in 15 rats. MAC's for methoxyflurane were 0.22 ± 0.05 per cent with the bulldog clamp in eight rats and 0.27 ± 0.03 per cent with the hemostat in 12 rats. (Key words: Halothane; Methoxyflurane; Minimum alveolar concentration; Anesthesia.)

FOR MAN, the dog, and the rat, accurate, reproducible assessment of depth of anesthesia is available, even when relatively blood-soluble anesthetic agents are used.[1-6] The pertinent data for laboratory animals, in particular the rat, largely refer to abolition of reflex responses to hemostat stimulus,[6] obviously a depth of anesthesia which affects many cardiovascular and microcirculatory mechanism(s). In animal studies requiring anesthesia, one either selects agents with minimal depressant effects and/or uses minimal amounts that result in "light" anesthesia. Where inhalation anesthesia is employed, there is an increasing need for quantitative information on depth of anesthesia. This paper describes a method for assessment of depth of anesthesia with inhalation anesthetics in the rat, using two gradients of stimulation and based on the concept and measurement of minimum alveolar concentration (MAC).

Material and Methods

Forty-three non-fasting, nonpregnant female Wistar CFN rats, weighing 100–160 g, were studied. The rectal temperature of each rat was measured with a calibrated thermistor (Yellow Springs Telethermometer) and the rat placed in a padded 1.5-liter container. Anesthesia was then induced by flushing the container at a flow rate of 1 l/min with oxygen containing 8 per cent halothane or 3 per cent methoxyflurane. Throughout the experiment, anesthesia of each rat was maintained with the same agent used for induction. When the righting reflex had been lost for 30–60 seconds, the animal was transferred to an operating platform. Anesthesia was then maintained with a conical face mask, using oxygen at 4 l/min with either 2–3 per cent halothane or 0.5–1 per cent methoxyflurane, respectively, sufficient to prevent movement in response to shaving or incision. The trachea was exposed with minimal blood loss, and tracheostomy performed, using polyethylene tubing (Intramedic PE-200 tubing, Clay Adams, N. Y.) 3 cm long. After this, controlled intermittent positive-pressure ventilation was instituted with a Harvard Rodent Respirator set at 1.5 ml/100 g body weight at a rate of 60–70 breaths/min,

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TABLE 1. MAC's for Halothane and Methoxyflurane at Two Levels of Stimulation* in the Rat

	Number of Rats	Stimulus Source*	MAC Per Cent		
			Mean	SD	Range
Halothane	8	Bulldog artery clamp	0.82	±0.12	0.65–0.95
	15	Hemostat	1.17	±0.51	1.07–1.22
Methoxyflurane	8	Bulldog artery clamp	0.22	±0.05	0.16–0.30
	12	Hemostat	0.27	±0.03	0.24–0.33

* Clamp or hemostat was applied at the base of the tail.

delivering 1–1.2 per cent halothane or 0.3–0.4 per cent methoxyflurane via a nonrebreathing system. This pattern of ventilation was chosen as it was found to be just sufficient to prevent attempts at spontaneous respiration; previous studies (unpublished data) showed that this method of ventilation resulted in Pa_{CO_2}'s of 25–35 torr, values similar to those seen in rats breathing spontaneously under light pentobarbital anesthesia. A rectal or esophageal lead from a thermostat controlling a warm-air blower (Air Curtain Incubator, Sage Instruments) was set to maintain esophageal temperature at 37 ± 0.1 C. All experiments were started about 10 AM and completed by 2 PM.

In 23 rats, the minimum alveolar concentrations (MAC) of halothane needed to prevent responses to two specific painful stimuli were determined. The stimuli consisted of application, in eight of the animals, of a bulldog artery clamp, and in 15 of a surgical hemostat, to the tail for 30 seconds or until a positive response, consisting of movement of the head or limbs, was elicited. End-tidal gas was obtained by briefly stopping the ventilator at the end of expiration and aspirating 0.2–0.3 ml from the tracheostomy tubing a 26-gauge needle into a 10-ml gas-tight syringe. The respirator was quickly restarted, and after 2–3 breaths the process was repeated, until 6–7 ml of end-tidal gas had been collected over about a minute. Preliminary studies of this technique showed that the gas obtained in this way had a P_{CO_2} of 5 ± 3 (SD) torr less than the arterial value, indicating that this gas is end-tidal. Halothane (expressed as per cent of one atmosphere) in both inspired and end-tidal gas was measured with a gas chromatograph (Perkin Elmer Vapor Fractometer). When

three successive end-tidal halothane samples over 15 minutes agreed to within 0.03 per cent, the rat's tail was clamped and the response noted. When there was no response after 30 seconds, end-tidal halothane was reduced. After this new end-tidal (alveolar) concentration had been kept constant for 15 minutes, the stimulus was again applied to the tail. This procedure was repeated until a positive response was elicited. The MAC was the mean of the last two alveolar concentrations or, in other words, the mean of the lowest concentration preventing and the highest permitting movement. MAC for methoxyflurane was then determined in the remaining 20 rats using the procedure just described with the bulldog clamp in eight and the hemostat in 12 animals.

Results

The means and standard deviations of MAC's for the two anesthetics, keeping rectal or esophageal temperature at 37.0 ± 0.1 C, are shown in table 1. Halothane MAC's were 0.82 ± 0.12 per cent when the bulldog clamp was applied as stimulus in eight rats, and 1.17 ± 0.51 per cent in 15 rats with the hemostat. Methoxyflurane MAC's for the same two stimuli were 0.22 ± 0.05 and 0.27 ± 0.03 per cent, respectively.

The data obtained from all experiments are shown in figure 1, in which the highest alveolar concentration of anesthetic that permitted movement is shown for each rat as a line extending up from the horizontal line, while the lowest concentration that prevented movement is shown as a downward line. In the halothane series, it can be seen that although there was a substantial range over which movement was unpredictably permitted or suppressed,
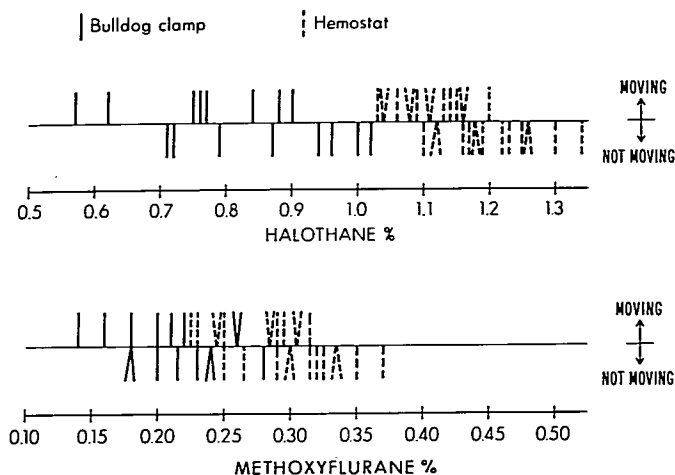
FIG. 1. MAC's for halothane and methoxyflurane in the rat. In each case the line extending up from the horizontal line represents the highest concentration of the anesthetic that prevents movement, while the lowest concentration that permits movement is shown as a downward line. *Solid line* = bulldog clamp stimulation; *broken line* = hemostat stimulation.

none of the eight rats moved when the bulldog clamp was applied (solid line) with alveolar concentrations of halothane greater than 0.9 per cent, and with alveolar concentrations of less than 0.7 per cent, no rat failed to respond to painful stimulation. The corresponding values were 1.2 per cent and 1.1 per cent, respectively, when the hemostat was used as a stimulus (broken line).

Similarly, in the methoxyflurane series it can be seen that with bulldog-clamp stimulation (solid line), no rat moved with an alveolar concentration greater than 0.26 per cent, while in none was movement on stimulation prevented by an alveolar concentration of less than 0.18 per cent. When hemostat stimulation was employed (broken line), none of the 12 rats moved with alveolar concentrations greater than 0.32 per cent methoxyflurane, and no rat failed to respond to stimulation with alveolar concentration less than 0.25 per cent.

Discussion

Since the introduction of the MAC concept, two definitions of MAC have been used: the first, the alveolar concentration needed to prevent response to a painful stimulus in 50 per cent of test subjects; the second, the alveolar concentration midway between the lowest concentration preventing and the highest permitting movement in response to a painful stimulus. The first definition is simpler to use, since it is easy to achieve one predetermined level of anesthesia (alveolar concentration) in each subject and therefore is readily applicable to man. However, while MAC determined using this method provides a useful indication of MAC for a population exposed to any given anesthetic, it does not define MAC for the individual. It can be seen from this and other studies that there is a distribution of individual MAC values about the species mean that covers a substantial range. It follows that when physiologic or pharmacologic processes are to be observed at various levels of anesthesia, for the MAC concept to be of value, MAC must be determined for each subject. For example, if MAC in one animal is 0.89 per cent halothane and in another, 0.68 per cent, then an alveolar concentration of, say, 1.8 per

cent, represents a depth of anesthesia of more than twice MAC in the first and nearly three times MAC in the second. Determination of MAC in each animal is a desirable goal and, as shown in this paper, one that can be achieved even in fairly young rats.

The difference between the values obtained in the present study for both halothane MAC and methoxyflurane MAC to preclude responses to painful stimuli derived from the bulldog clamp and the surgical hemostat, respectively, is not surprising. The deeper tail compression and greater trauma derived from the application of the latter instrument are likely to result in greater recruitment of deep sensory fibers than would occur with the former. It is of interest that the halothane MAC (1.7 ± 0.51 per cent) needed in the present study to preclude responses to painful stimulation of the tail by a hemostat is of about the same magnitude as that reported for Sprague-Dawley rats (1.33 ± 0.41 per cent)[6] for the same stimulus source. In the latter study, using older animals, MAC was determined from the inspired concentration of halothane after approximate equilibrium with alveolar levels had occurred.

The method described permits the measurement of end-tidal anesthetic concentrations without undue difficulty, and avoids the circumstances that are known to alter MAC, such as other anesthetic or depressed drugs, temperature change, hypoxia, or hemorrhage.[1] It is applicable to rats undergoing other experiments under anesthesia; for example, laparotomy can be performed where depth of anesthesia to a selected stimulus can be specifically defined.

We believe that this method of determining depth of anesthesia in the rat is a valuable, readily applicable, noninvasive technique suitable for the continuing study of the multifaceted effects of inhalational anesthetic agents.

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Obstetrics

THERAPEUTIC ABORTION WITH PROSTAGLANDINS Therapeutic abortions in early and mid-gestation are becoming extremely popular in many parts of the United States. A new technique requiring no anesthesia is described, consisting of intra-amniotic injection of either prostaglandin F_2-alpha or prostaglandin E_2 in mid-pregnancy. Termination of pregnancy was successful in 11 of 13 women when prostaglandin E_2 alone was used, and successful in only six of 14 when prostaglandin F_2-alpha was used. The technique is simple and appears to be free of serious side-effects. (*Roberts, G., and others: Therapeutic Abortion by Intra-amniotic Injection of Prostaglandins. Br. Med. J. (7 Oct.): 12–14, 1972.*)