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Anesthetic Potencies of Isoflurane, Halothane, and Diethyl Ether for Various End Points of Anesthesia

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In experiments with rats, the authors compared potency ratios and slopes of dose-effect curves of isoflurane, halothane, and diethyl ether for three end points of anesthesia: loss of righting reflex (RR), abolition of purposeful movement (PM) response to painful stimuli, and abolition of heart rate (HR) response to painful stimuli. Determinations of potency were based on the direct measurement of brain concentrations of anesthetics with the use of gas chromatography.

It was found that the ratio of the PM ED50 to RR ED50 was 2.41 for isoflurane, 1.74 for halothane, and 1.25 for diethyl ether. They were significantly different for all three agents. Differences between the slopes of the dose-effect curves for RR and PM were significant only with diethyl ether (7 *vs.* 28). The ratios of HR ED50 to PM ED50 were not significantly different for the studied agents and there were no differences found between the slopes of the dose-effect curves for PM and HR. The results suggest that heart rate response to a noxious stimuli in contrast to the righting reflex is depressed by inhalation anesthetics through a mechanism similar to that underlying the depression of purposeful movement response to a noxious stimuli. Heart rate response to a noxious stimuli might be used as an alternative index for the measurement of anesthetic potency. (Key words: Anesthetics, volatile: diethyl ether; halothane; isoflurane. Potency, anesthetic: dose-response.)

SHIM AND ANDERSEN[1] reported that the fraction of MAC which abolished the righting reflex in toads and mice varied significantly for different inhalation anesthetics. Deady *et al.*[2] recently found that the ratios of MAC to ED50 for abolishment of the righting reflex were different, as determined in halothane- and isoflurane-exposed mice. The authors concluded that, at lease in part, the righting reflex is depressed by a different mechanism from that which depresses the response to painful stimuli.[2] This may provide a basis for two alternative suggestions. First, that the effect on the righting reflex does not represent an "anesthetic" effect, but rather a side effect perhaps on the vestibular apparatus. Second, that the state of anesthesia includes various components of anesthesia and the loss of righting reflex represents a component which is quite different from the component represented by the blockade of movement response to painful stimulation.

Although abolition of the righting reflex is accepted as a standard measure of anesthesia, it is certainly not one of the requirements for clinical anesthesia. By contrast, blockade of cardiovascular responses to surgical stimulation is a clinically essential element of anesthetic action. Whether inhalation anesthetics act on cardiovascular and motor responses to painful stimuli through a similar mechanism is of potential importance.

The purpose of the present study was to examine whether cardiovascular (autonomic) and motor (somatic) responses to painful stimuli are depressed by inhalation anesthetics through similar or different mechanisms. To achieve this goal, we used, as the end points of anesthesia, abolition of heart rate response to painful stimuli,[3] and compared the effects of isoflurane, halothane, and diethyl ether on this index with their effects on two other indices of anesthetic action: abolition of movement response to painful stimuli, and loss of righting reflex. We tried to find an answer to the above question by using the two following approaches: 1) assessment of ratios of ED50 for one end point to another. If these ratios varied from agent to agent, it would be evidence for the absence of a constant proportionality between various actions on the central nervous system and not consistent with a unitary theory of narcosis. 2) Evaluation of the slopes of dose-effect curves at different end points of anesthesia for the same agent. It is commonly held that parallel dose-effect curves are indicative of the identity of the mechanism of action.[4] If the heart rate response is depressed by an anesthetic through a different mechanism than that which is involved in depression of movement response to painful stimuli or loss of righting reflex, respective dose-effect curves for the anesthetic would have different slopes.

Methods

We used 334 male Sprague-Dawley rats (300–350 g). Experiments were performed from midmorning to midafternoon to minimize the possibility of circadian variation. The rats were anesthetized and kept in a clear chamber with the rat's tail protruding from a special

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opening. Anesthetic–oxygen mixture was directed into the chamber at a rate of 4 l/min. Halothane and isoflurane were vaporized in a Draeger vaporizer and the level in the chamber was monitored with a calibrated Engstrom Emma gas analyzer. Diethyl ether was vaporized in a copper kettle vaporizer. Rat colonic temperature was monitored (Yellow Springs Inst. Co. UI 43) and maintained at 37.0° C with a heating pad. Each rat was exposed to only one predetermined concentration of anesthetic for 30 min (60 min for diethyl ether), then presence or absence of the end point of anesthesia was determined. The rat was killed while in the chamber by placing a clamp around the neck. The whole brain was removed, homogenized, and transferred to preweighed, capped tubes containing carbon tetrachloride and chloroform.[5,6] After extraction, tissue anesthetic concentration was determined by gas chromatography.[7]

The following end points of anesthesia were used: 1) loss of righting reflex[8]: The test was regarded as positive if a rat failed to right itself for 15 s. 2) Prevention of purposeful movement in response to a noxious stimuli[9]: The animals were stimulated for 60 s by placement of a 1-kg weight on the middle of the tail (pressure surface of 0.25 cm^2). Only the purposeful movement of the head or legs was considered to be a response. Stiffening, coughing, hyperventilating, or vocalizing were not considered. 3) Prevention of the rise in heart rate in response to a noxious stimuli[3]: Stimulation was the same as for the movement response. The heart rate was derived from an ECG. A cardiotachometer triggered by ECG signals provided records of heart rate on a Grass 7-D polygraph. An increase in heart rate of greater than 1% was regarded as a positive response. Without anesthesia, the heart rate increase was 12.5 ± 1.0% (n = 7). Baseline (resting) heart rate of anesthetized rats did not significantly influence the degree of heart rate response to a noxious stimuli (fig. 1).

With each of the three anesthetics, three series of experiments were performed: for the righting reflex, purposeful movement response, and heart rate response. In each series of experiments, five to six groups of rats were used consisting of five to seven rats each. In one group of animals, the inspired concentration of the agent was low enough so that all animals were unaffected, and in another group, it was high enough so that all were affected. In the three to four remaining groups, the concentrations of the agent were spaced equally between the abovementioned doses. In each group, individual values of anesthetic brain concentration and presence of the response were obtained. These responses and anesthetic brain concentrations were used to generate dose-effect curves. To achieve this, we applied logit quantal analysis which constructs the curve directly from individual observations.[10] All calculations

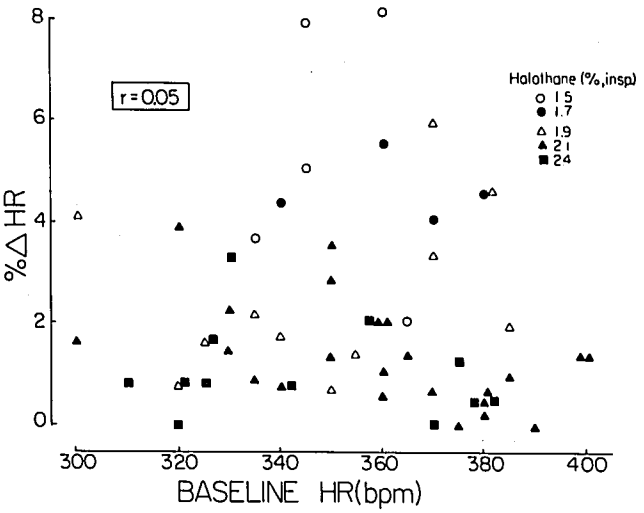

FIG. 1. Relationship of heart rate increase (to a noxious stimuli) to baseline heart rate in rats anesthetized with halothane. Note absence of any significant influence of baseline heart rate on the degree of the heart rate response to a noxious stimuli (r = 0.05).

were performed using a logit procedure from SAS[11] on an IBM 370 computer.

The following data were derived: (1) Median values of anesthetic brain concentration-effect curves: In order to use conventional statistical notation, they are abbreviated as ED50 (RR ED50 for righting reflex, PM ED50 for purposeful movement response, and HR ED50 for heart rate response). ED50 differences were tested using a *t* test. Potency ratios PM ED50/RR ED50 and HR ED50/PM ED50 for isoflurane, halothane, and diethyl ether were calculated. They were tested using analysis

TABLE 1. Median Effective Brain Concentrations of Isoflurane, Halothane, and Diethyl Ether for Different End Points of Anesthesia

	n	End Point	ED50 [mg/100 g (brain)]
Isoflurane	30	RR	8.2 (7.2–9.0)*
	30	PM	19.8 (18.7–20.9)*
	34	HR	34.4 (31.4–37.1)*
Halothane	36	RR	15.5 (13.5–18.0)*
	29	PM	26.9 (23.6–29.1)*
	40	HR	42.2 (38.7–45.4)*
Diethyl ether	36	RR	65.9 (56.5–74.4)*
	39	PM	82.3 (78.1–85.8)*
	42	HR	114.4 (84.6–156.4)†‡

RR = righting reflex; PM = purposeful movement response to painful stimuli; HR = increase in heart rate in response to painful stimuli.
n = number of animals used to determine dose-response curve.
Numbers in parentheses indicate 95% fiducial limits, † 90% fiducial limits.
Asterisks and double dagger denote statistical significance in the difference between ED50 values for the three series of experiments (RR, PM, HR) with the same agent, * $P < 0.001$, ‡ $P < 0.05$.

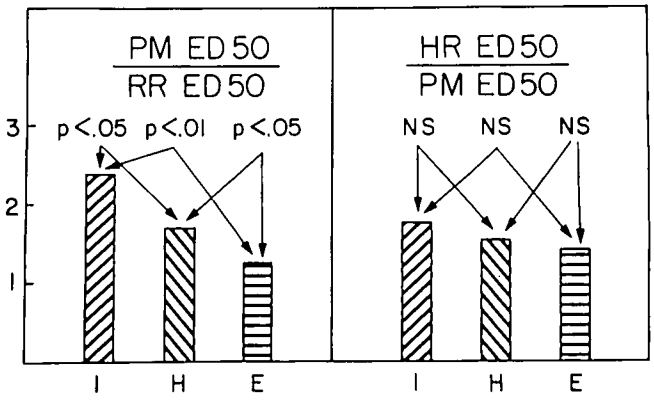

FIG. 2. Potency ratios of isoflurane, halothane, and diethyl ether for different end points of anesthesia. PM ED50 = median effective anesthetic brain concentration for loss of righting reflex. RR ED50 = median effective anesthetic brain concentration for abolition of purposeful movement response to painful stimuli. HR ED50 = median effective anesthetic brain concentration for abolition of increase in heart rate in response to painful stimuli. Variation of PM ED50/RR ED50 ratio for different anesthetics suggests that the righting reflex is abolished by a different mechanism from that which abolishes response to painful stimulation.

of variance of the linear combination of the logarithms of the ED50s. Estimates of log ED50 and its variance were obtained from the parameters of the logistic regression using Fieller's theorem.[12] (2) The slopes of anesthetic brain concentration-effect curves for the above end points of anesthesia (logit units/$\log_e$-brain concentration): Slope differences were tested using a t test.

Results

Table 1 shows ED50 values based on anesthetic brain concentrations. With all three studied agents, ED50 values for righting reflex were smaller than those for purposeful movement response, and for purposeful movement, smaller than for heart rate response. Quantitative relationships between potencies of the anesthetics for various end points of anesthesia are presented in figure 2. The ratio of PM ED50 to RR ED50 was 2.41 for isoflurane, 1.74 for halothane, and 1.25 for diethyl ether. Differences between these three ratios were statistically significant. The ratio of HR ED50 to PM ED50 was 1.74 for isoflurane, 1.57 for halothane, and 1.39 for diethyl ether. Differences between these ratios were not statistically significant.

Slopes of the dose-effect curves for different end points of anesthesia are shown in figures 3–5. A statistically significant difference in slopes ($P < 0.05$) was found only between the righting reflex and purposeful movement response for diethyl ether. The diethyl ether heart rate response curve is not presented because of our inability to make reliable observations at the lower and upper ends of the curve. Since the upper portion of the purposeful movement response curve overlapped the lower portion of the heart rate response curve, movement in response to painful stimuli prevented proper recording of the heart rate response. With a high concentration of diethyl ether, depression of respiration was too prominent to rely on the obtained values.

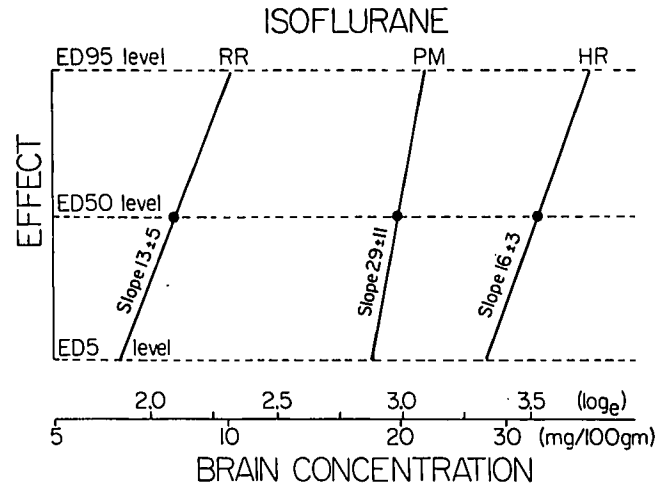

FIG. 3. Isoflurane brain concentration-effect curves. RR = loss of righting reflex; PM = prevention of purposeful movement in response to a noxious stimuli; HR = prevention of heart rate increase in response to a noxious stimuli.

Discussion

The brain concentrations of the anesthetics obtained in our study were similar to those previously found by Wolfson *et al.*[5] In their experiments, at the level of anesthesia preventing movement response to painful stimulation, brain concentrations of halothane and isoflurane were 27.8 ± 0.6 mg/100 g, and 15.5 ± 0.5 mg/100 g, respectively. In our study, PM ED50 was

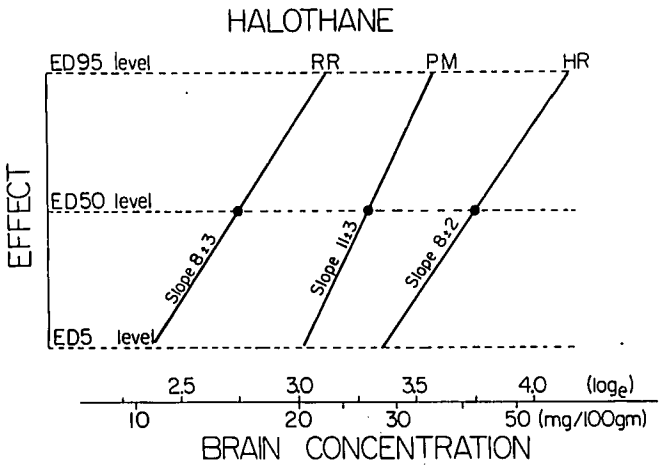

FIG. 4. Halothane brain concentration-effect curves.

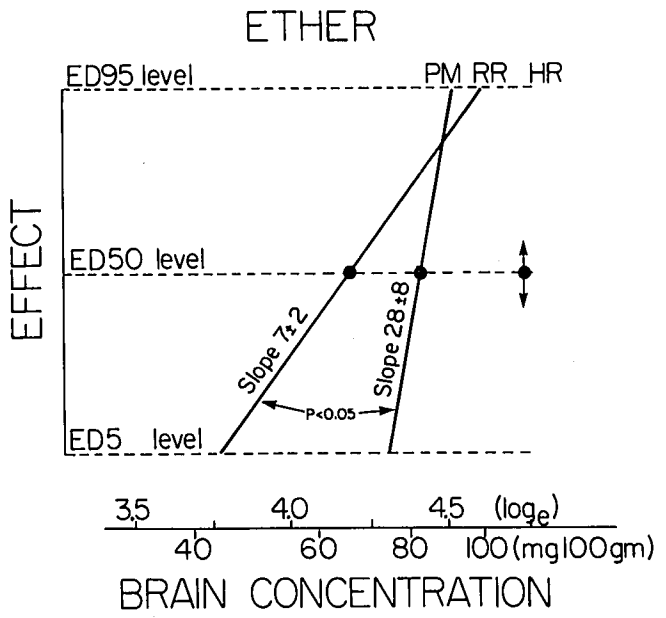

FIG. 5. Diethyl ether brain concentration-effect curves. The explanation for the lack of HR response values is in the text.

26.0 mg/100 g (95% fiducial limits: 23.6 –29.1 mg/100 g) for halothane, and 19.8 mg/100 g (95% fiducial limits: 18.7–20.9 mg/100 g) for isoflurane.

Our results confirmed the suggestion that the righting reflex is depressed by a different mechanism from that which depresses movement response to painful stimulation. In table 2, our data for motor response-righting reflex potency ratios are compared with the ratios obtained for similar end points by other investigators. Despite methodological differences (different species, inspired or brain concentrations of anesthetics), values reported in the three compared studies are similar. The ratio of MAC (PM ED50) to RR ED50 for isoflurane was greater than that for halothane (Deady *et al.*[2]; this study). For halothane, this ratio was greater than that for diethyl ether (Shim and Andersen[1]; this study). The RR ED50 was not a predictable fraction of MAC (PM ED50) with different anesthetics. Differences between the slopes of the dose-effect curves for righting reflex and movement response were statistically significant only with diethyl ether. The mean numerical difference between isoflurane righting reflex and isoflurane purposeful movement response curves was pronounced (13 ± 5 *vs.* 29 ± 11), but nevertheless statistically insignificant. Possibly the difference in the slopes of dose-effect curves is a less sensitive index for testing the identity of the mechanism of action than agent to agent variability in the potency ratios. Of interest is the observation that the PM slopes tended to be steeper than RR slopes, perhaps indicating an effect on a more homogeneous site of action.

With all three agents studied, heart rate response ED50 had a relatively constant ratio to the concentration necessary to prevent purposeful movement response to painful stimulation (PM ED50). There were also no differences in the slopes of dose-effect curves for motor response and heart rate response. The obtained results suggest that cardiovascular (autonomic) and motor (somatic) responses to painful stimuli are depressed by inhalational anesthetics through similar mechanisms.

The constant ratio of the HR ED50 to the PM ED50, of approximately 1.5, suggests that tachycardia in response to surgical stress might be suppressed by an anesthetic concentration that is 50% above the concentration depressing movement response. There is evidence that these results might be transferable to humans. Roizen *et al.*[13] have shown in patients that ED50 for blocking an increase in blood norepinephrine upon skin incision was 1.4 MAC for halothane–nitrous-oxide, and 1.6 MAC for enflurane–nitrous-oxide. It is quite possible that at these concentrations, an increase in heart rate caused by hemorrhage or hypoxia, also will be blocked. Duke *et al.*[14] have shown that halothane completely blocks heart rate response to baroreceptor stimulation at 1.25 MAC. However, only comparison of the effects of anesthetics on heart rate response to noxious and baroreceptor stimulations under similar conditions can provide an answer as to which of the responses is more sensitive to inhalation anesthetics. A constant ratio of the HR ED50 to the PM ED50 also suggests that heart rate response to a noxious stimuli could be used as an alternative index for the measurement of anesthetic potency.

Our data regarding anesthetic action on righting reflex and purposeful movement response conflict with the unitary theory of anesthesia. Nevertheless, they might be explained within the framework of the theory. It is possible to suggest that the hydrophobic factor, through which inhalation anesthetics are acting, has slightly different properties to the particular neural

TABLE 2. Ratios of Inhalation Anesthetic Concentrations that Abolish Purposeful Movement Response to Concentrations that Abolish Righting Reflex

	MAC(PM ED50) / RR ED50		
	Shim and Andersen*[1]	Deady *et al.*†[2]	This Study‡
Isoflurane	——	2.23	2.41
Halothane	1.34	1.68	1.74
Diethyl ether	1.13	——	1.25

* Toads, inspired anesthetic concentrations.
† Mice, inspired anesthetic concentrations.
‡ Rats, brain anesthetic concentrations.

pathway concerned, and these differences are more pronounced between the pathways for the righting reflex and the movement response than between pathways for the movement response and the heart rate response (the latter pathways have a common afferent part).

In conclusion, abolition of heart rate response to a noxious stimulus (HR ED50) in contrast to the loss of righting reflex (RR ED50) has a constant ratio to MAC (PM ED50). This may indicate that cardiovascular (autonomic) and motor (somatic) responses to noxious stimuli are depressed by inhalation anesthetics through similar mechanisms. Heart rate response to a noxious stimuli could possibly be used as an alternative index for the measurement of anesthetic potency.

References

1. Shim CY, Andersen NB: Minimal alveolar concentration and dose-response curves in anesthesia. ANESTHESIOLOGY 36:146–151, 1972
2. Deady J, Koblin DD, Eger EI, D'Aust B, Heavner JE: Anesthetic potencies and the theories of narcosis. Anesth Analg (Cleve) 59:535, 1980
3. Kissin I, McGee T, Smith LR: The indices of potency for intravenous anaesthetics. Can Anaesth Soc J 28:585–589, 1981
4. Goldstein A, Aronow L, Kalman SM: Principles of Drug Action: The Basis of Pharmacology, Second edition. New York: John Wiley and Sons, 1974, p 77
5. Wolfson B, Kielar CM, Lake C, Hetrick WD, Siker ES: Anesthetic index—a new approach. ANESTHESIOLOGY 38:583–586, 1973
6. Wolfson B, Hetrick WD, Lake C, Siker ES: Anesthetic indices—further data. ANESTHESIOLOGY 48:187–190, 1978
7. Wolfson B, Ciccarelli HE, Siker ES: Gas chromatography using an internal standard for the estimation of ether and halothane levels in blood. Br J Anaesth 38:591–595, 1966
8. Junssen PAJ, Neimegeers CJE, Marsboom RPH: Etomidate, a potent non-barbiturate hypnotic. Intravenous Etomidate in mice, rats, guinea-pigs, rabbits and dogs. Arch Int Pharmacodyn 214:92–132, 1975
9. Eger EI II: Anesthetic Uptake and Action. Baltimore, Williams and Wilkins 1974, p 2
10. Waud DR: On biological assays involving quantal responses. J Pharmacol Exp Ther 183:577–607, 1972
11. SAS Institute, SAS Supplemental Library User's Guide. Cary, SAS Institute, 1980, p 83
12. Finney DJ: Probit Analysis, Third edition. Cambridge, Cambridge University Press, 1971, p 78
13. Roizen MF, Horrigan RW, Frazer BM: Anesthetic doses blocking adrenergic (stress) and cardiovascular responses to incision—MAC BAR. ANESTHESIOLOGY 54:390–398, 1981
14. Duke PC, Fownes D, Wade JG: Halothane depresses baroreflex control of heart rate in man. ANESTHESIOLOGY 46:184–187, 1977