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Minimal Alveolar Concentration (MAC) and Dose–Response Curves in Anesthesia

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Toads and mice were anesthetized with chloroform, cyclopropane, diethyl ether, divinyl ether, fluroxene, halothane, methoxyflurane, or trichloroethylene in order to compare the concentrations necessary to abolish the righting reflex, the motor response to mechanical stimuli (MAC), respiratory activity, and cardiac function. All four responses could be reproduced within narrow concentration ranges of each anesthetic. However, these concentrations were not the same fraction of MAC with different anesthetics. Thus, with chloroform 1 MAC and with trichloroethylene 0.65 MAC was necessary to abolish the righting reflex in toads. It is proposed that anesthesia involves a number of different effector sites. The authors conclude that the effect at MAC represents the sum of points on two or more dose-response curves. (Key words: Toads; Mice; General anesthetic agents; Righting reflex; Respiratory arrest; Cardiac arrest; MAC.)

THE MINIMAL ALVEOLAR CONCENTRATION (MAC) has provided us [1, 2] and others [3] with a convenient measurement in the bioassay of anesthetic agents. MAC represents the dose that abolishes the motor response to a standard stimulus. The response, however, is the result of activity in a multitude of areas: receptor organs, nerve axons, peripheral synapses, nerve cell bodies, CNS synapses, motor endplates, etc. All components of the response may be influenced by anesthetics.[4] Just as local anesthesia or muscle relaxants would modify MAC by specific actions, so might one anesthetic gas abolish the motor response by affecting one area in the system, while another anesthetic might spare that area and preferentially affect a different one.

In order to demonstrate the variation among anesthetics in their biases for certain areas, we compared the concentrations of different agents necessary to abolish the righting reflex, the motor response to mechanical stimuli, respiratory activity, and cardiac function.

Method

Healthy toads (*Bufo marinus*) and mice (Swiss albino) were numbered and kept in the laboratory for months. Only animals from this acclimatized group were used for the experiments.

Eight general anesthetic agents were studied: chloroform, cyclopropane, diethyl ether, divinyl ether, fluroxene, halothane, methoxyflurane, and trichloroethylene. The concentrations that 1) abolished the righting reflex, 2) prevented reaction to a standard mechanical stimulus, and produced 3) respiratory and 4) cardiac arrest were recorded. All anesthetic concentrations were asssesed by gas chromatography in the inspired mixture at equilibrium except the measurements at cardiac arrest, which were done using end-expiratory samples. The times to attainment of equilibrium between inspired and expired concentrations at MAC had been determined in a previous study [1] or in pilot experiments. We kept the inspiratory concentrations constant for twice as long as necessary for equilibrium at or below MAC and four times as long above MAC. The righting reflex was considered lost when the animals lay down and were unable to stand up on prodding. MAC was the concentration that prevented any motor reaction to the pressure of a clamp applied for 2 seconds to a lower extremity. The clamp pressure did not break the skin, but additional pressure did not increase MAC.[1] Respiratory arrest was recorded at fewer than 3 breaths/

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TABLE 1. Equilibrated Inspiratory (or End-expiratory for Cardiace Arrest) Concentrations of Eight Anesthetics necessary to Abolish Righting Reflex, Response to Clamp Pressure (MAC), Respiration and Cardiac Function in the Toad*

	Concentration (Vol Per Cent)				
	Righting Reflex	MAC	Respiratory Arrest	Cardiac Arrest	Convul-ions
Cyclopropane	6.3 ± 0.05	9.0 ± 0.02	13.8 ± 0.25	>80	
Diethyl ether	1.49 ± 0.03	1.69 ± 0.02	3.02 ± 0.02	15.5 ± 0.47	
Fluroxene	2.8 ± 0.06	3.4 ± 0.07	4.7 ± 0.10	19.9 ± 0.70	
Halothane	0.53 ± 0.01	0.71 ± 0.01	0.86 ± 0.01	3.8 ± 0.12	
Chloroform	0.41 ± 0.01	0.41 ± 0.01	0.52 ± 0.02	2.0 ± 0.07	
Divinyl ether	2.73 ± 0.06	3.69 ± 0.03	8.17 ± 0.20	14.1 ± 0.45	8.16 ± 0.22
Methoxyflurane	0.17 ± 0.01	0.22 ± 0.01	0.27 ± 0.01	0.62 ± 0.01	
Trichloroethylene	0.13 ± 0.01	0.20 ± 0.01	0.26 ± 0.07	0.44 ± 0.02	0.22 ± 0.01

* Concentrations of agents that consistently produce convulsions are included. Means of five experiments ± SE.

min, cardiac arrest at fewer than 5 beats/min by ECG.

Approximate anesthetic concentrations necessary to produce these four levels were determined in pilot experiments. The concentration that produced respiratory arrest was assessed as follows: equilibration at MAC was achieved in a toad, whereupon anesthesia was deepened and equilibration at increments of 1/5 MAC was continued until respiration ceased.

For the actual study, a toad or a mouse was placed in a 2-liter jar at room temperature. The jar was aerated with the anesthetic in oxygen, 8 l/min, and equilibration with an ambient anesthetic concentration well below the concentration necessary to abolish the righting reflex was attained. Anesthesia then was deepened and equilibration at increments of about 1/10 MAC continued until the righting reflex was lost. At this point, anesthesia was lightened to the previous level and deepened again. When deepening and lightening of anesthesia were associated with appropriate disappearance and recurrence of the reflex, the righting reflex in that animal was considered lost at the mean of the two concentrations. MAC was determined the same way, with no reaction to clamp pressure as the endpoint.

Next, equilibration with an inhaled concentration well below that known to produce respiratory arrest was achieved in the toads. Anesthesia was deepened, equilibration in steps of 1/10 MAC was accomplished as before, and the smallest inhaled concentration that depressed respiration to the point of 3 or fewer breaths/min was recorded. At this time the animals were removed from the jar, the tracheas were intubated, and they were ventilated as previously described.[1] The anesthetic concentrations of end-expiratory and inspiratory samples were determined, and a constant ratio between the two was taken to indicate equilibration.[1, 5] Anesthesia was deepened and equilibration attained as described above until heart rate had decreased to fewer than 5 beats/min. Anesthesia then was lightened to the previous level and deepened again; by lightening and deepening anesthesia, we determined the concentration at which heart rate approximated 5 beats/min ("cardiac arrest").

The experiments with mice were identical, but only the concentrations of chloroform and cyclopropane necessary to abolish the righting reflex and the response to clamp pressure were studied.

All of the animals recovered and were used randomly for several experiments. There were five animals in each group. All results given are mean ±SE.

Results

The findings in the toad are listed in table 1 and illustrated in figures 1 and 2. The righting reflex was lost either at or below MAC. MAC always was reached before respiratory arrest, and respiratory arrest always before cardiac arrest. Cyclopropane, 80 per cent,

did not cause cardiac arrest. The concentration necessary for a certain effect was remarkably reproducible from animal to animal, as demonstrated by small standard errors. Trichloroethylene was the most potent, and cyclopropane the least potent, agent. Trichloroethylene exerted the effects studied over a threefold range, cyclopropane over at least a 13-fold range.

Figures 1 and 2 show that none of the anesthetic effects chosen as endpoints had constant interrelationships. Thus, with chloroform 1 MAC, and with trichloroethylene 0.65 MAC, was needed to abolish the righting reflex.

The results in mice are shown in table 2. The concentrations of chloroform and cyclopropane necessary to prevent the righting reflex and the response to clamp pressure were higher in mice than in the toad, but the numerical relationship between the concentrations was the same in toad and mouse.

All toads anesthetized with trichloroethylene or divinyl ether developed intermittent tonic and clonic convulsions or athetoid movements at consistent levels of anesthesia (table 1). With trichloroethylene these convulsions appeared just beyond MAC; with divinyl ether, at respiratory arrest. None of the other agents had similar effects. With deepening anesthesia and decreasing heart rate, the ECG always showed decreasing voltage.

Discussion

This study attempts to answer questions about anesthetic levels that do not permit endotracheal intubation. Consequently, we have reported inspiratory rather than expiratory anesthetic concentrations. Approximate equilibration with the inspiratory concentration was considered to have been attained because: 1) We previously determined[1] the times to attainment of constant ratios approaching unity between inspiratory and end-expiratory concentrations in toads with a number of agents. Twice these times were allowed for equilibration at light, four times as long at deep, anesthesia. 2) MAC's in this study correlated well with findings in a previous study.[1] 3) All results were remarkably reproducible, as indicated by very small standard errors. 4) At the end of each toad experiment, when cardiac arrest was the anesthetic endpoint, determinations of concentrations of inspiratory and end-expiratory gases confirmed the validity of our assumption about equilibration time. 5) Our findings in mice also agree with reports by others. Epstein *et al.*[6] found that chloroform, 6.4 mm Hg, abolished the righting reflex in 50 per cent of mice, and Munson *et al.*[7] found cyclopropane MAC in rats to be 15.6 vol per cent. Thus, we accepted all results listed as reasonable expressions of the

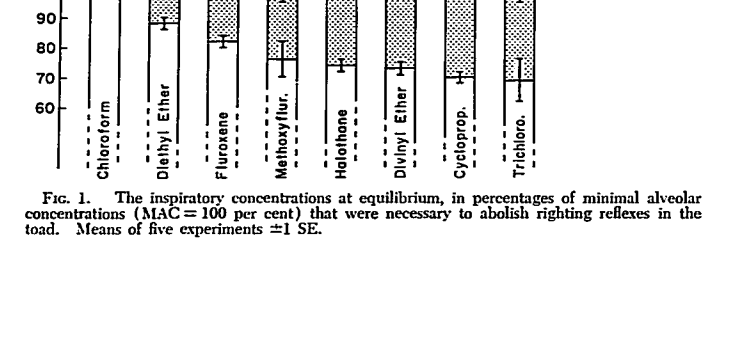

FIG. 1. The inspiratory concentrations at equilibrium, in percentages of minimal alveolar concentrations (MAC = 100 per cent) that were necessary to abolish righting reflexes in the toad. Means of five experiments ±1 SE.

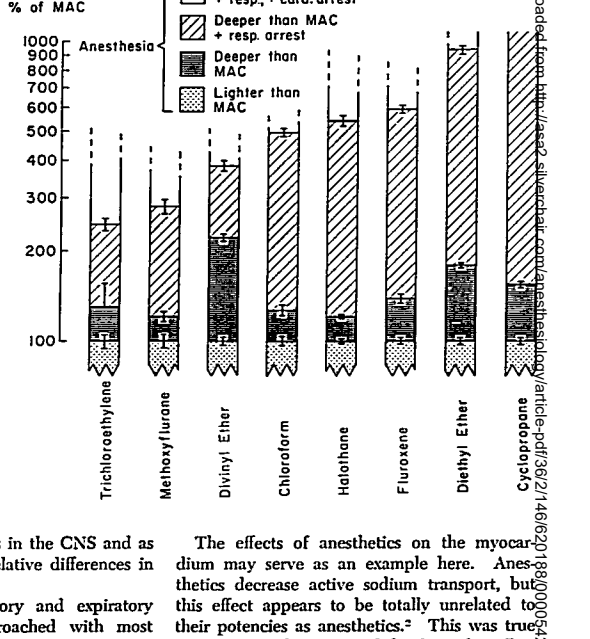

FIG. 2. The inspiratory or end-expiratory (cardiac arrest) concentrations, in percentages of minimal alveolar concentrations (MAC = 100 per cent) that abolished respiratory and cardiac function in toads. Means of five experiments ± 1 SE.

partial anesthetic pressures in the CNS and as good expressions of the relative differences in CNS partial pressures.

Unity between inspiratory and expiratory gases probably was approached with most agents tested, but the degrees of equilibration cannot fully be ascertained, unless expiratory as well as inspiratory gas concentrations are measured. However, we wished only to assess whether different anesthetics depress nerve functions in a parallel progression. The study supplied a clear answer to this question: with some anesthetics two different endpoints were reached simultaneously; with others, these two endpoints were attained at widely different concentrations. These differences were unrelated to the solubility in blood of the anesthetics. None of the concentrations necessary to produce the anesthesia levels chosen as endpoints showed constant relationships. The responses to anesthesia in two species changed with the agent used. A number of different effector sites must have been involved, and the sensitivities of these sites to anesthetics must have varied from agent to agent.

The effects of anesthetics on the myocardium may serve as an example here. Anesthetics decrease active sodium transport, but this effect appears to be totally unrelated to their potencies as anesthetics.[2] This was true whether anesthesia was defined as the effect on reflex activity, motor response to mechanical stimuli, or respiratory effort. However, the effect on sodium transport may be correlated with an effect on the myocardium. The concentrations necessary to produce cardiac arrest with cyclopropane, ethyl ether, halothane, and methoxyflurane in this study coincided with

TABLE 2. Inspiratory Concentrations at Equilibrium in Mice That Prevented Righting Reflexes and the Response to Clamp Pressure (MAC)

	Concentration (Vol Per Cent)	
	Righting Reflex	MAC
Chloroform	0.72 ± 0.01	0.72 ± 0.01
Cyclopropane	12.6 ± 0.29	17.7 ± 0.47

TABLE 3. End-expiratory Concentrations at Equilibrium That Produced Cardiac Arrest in the Toad *in vivo*, and Approximate Concentrations That Inhibited Active Sodium Transport to 66 Per Cent of Normal in the Toad Bladder *in Vitro*[2]

	Effective Concentration (Vol Per Cent)	
	33 Per Cent Na^+ Transport Depression	Cardiac Arrest
Cyclopropane	78.0	>80
Ether	13.0	15.5
Halothane	4.0	3.8
Methoxyflurane	0.6	0.6

the concentrations of the same agents necessary to produce about 33 per cent inhibition of active sodium transport in the toad bladder.[2] Only these four agents were used in both studies (table 3). The concentrations depressing sodium transport by 33 per cent were obtained by interpolation, since this particular degree of inhibition was not the principal subject under study. A relationship between effects on sodium transport and myocardial function was predicted earlier,[2, 3] but a numerical relationship is no proof of a direct link. It does, however, focus our attention on the possibility that one is cause and the other, effect. Perhaps anesthetics depress myocardial function through an effect on sodium transport and produce unconsciousness at an entirely different site. The potency at one effector site would not need to mirror potency at another site.

MAC was not a predictable fraction or multiple of the concentration necessary to prevent the righting reflex or any other endpoint studied. This finding may be interpreted in several ways. Our explanation is illustrated in figure 3, which shows dose–response curves for three imaginary anesthetics. It is assumed that the response at MAC is the sum of reflex depression and depression of pain sensation. With agent A the unresponsiveness recorded at MAC is the result of 75 per cent reflex depression, 25 per cent analgesia. Agent B achieves this effect with 50 per cent reflex depression and 50 per cent analgesia. Agent C combines 75 per cent analgesia and 25 per cent reflex depression in MAC. In the study, agent A could represent trichloroethylene; agent B, fluroxene; agent C, chloroform. According to this scheme, trichloroethylene would have a weaker, and chloroform a stronger, analgesic effect at lower concentrations. However, it is important to state that we looked for only two—and found circumstantial evidence for two—effects that combine to prevent the motor response to clamp pressure. "Reflex depression" and "analgesia" may prove each to incorporate several components, and other systems that contribute to the effect recorded at MAC may be described.

The findings in the toad with lower concentrations of chloroform and cyclopropane were corroborated in mice. We assume that similar relationships between effects of anesthetics on reflex activity and motor responses to mechanical stimuli exist in other species. Differences in concentrations necessary for attainment of a certain response may be attributed to species differences.

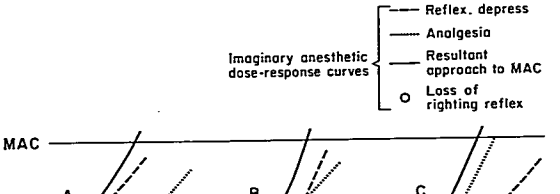

FIG. 3. Hypothetical curves showing that the unresponsiveness to clamp pressure at MAC may result from a combination of analgesia and depressed reflex activity with three different imaginary anesthetic agents. The effect at MAC with agent A coniststs of ¾ analgesia, ¾ reflex depression; with agent B, ½ of each; with agent C, ¾ analgesia and ¼ reflex depression. Since the righting reflex disappears at a certain level of reflex depression, it would disappear well below MAC with agent A and at MAC with agent C.

Waud and Waud[9,10] stated that MAC represents only one point on a dose–response curve, and that the dose–response curves for various anesthetics may be different, limiting the value of MAC as a research tool. Eger[11] did not fully share this view. The problem is quite complex. We suggest that MAC represents points on two or more dose–response curves and is the sum of two or more effects. Accordingly, it is not justifiable to use MAC for speculation about mechanisms of action.

Cardiac arrest always followed respiratory arrest, and both were consistently produced within narrow concentration ranges. The margin of safety indicated by these results applies to the toad only, since the tendency of the toad myocardium to fibrillate at room temperature is small.[12] Trichloroethylene and divinyl ether always produced convulsions at certain high concentrations. Divinyl ether frequently, and trichloroethylene less frequently, produce abnormal motor activity in man as well.[13] In our animals the degrees of motor activity found ranged from moderate athetoid movements to frank clonic convulsions. However, these were intermittent and probably did not significantly alter the data obtained with the two agents.

We cannot explain why these two agents produced convulsions, but are reminded that flurothyl may be first a convulsant and second an anesthetic.[14] In a study of thiopental and a non-anesthetic, convulsant analogue of thiopental, butyl crotyl thiobarbituric acid (BCT), we found with low concentrations that the former decreased and the latter increased the permeability of toad bladder to sodium.[15,16] This was the only difference between the two agents in their effects on passive and active sodium transport. At high concentrations both increased permeability, but thiopental much less than BCT. Increased sodium permeability may prove to be yet another effect shared by anesthetics, and trichloroethylene and divinyl ether may prove to be particularly potent in this area.[17]

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