The Effect of Oxygen on Minimal Anesthetic Requirements in the Toad

Chi Yun Shim, M.D., and Nikaan B. Andersen, M.D.†*

Minimal anesthetic requirements for cyclopropane, nitrous oxide, thiopental, ether, halothane, and methoxyflurane in the toad were studied. Requirements in the toad at room temperature were similar to those in the dog and in man at body temperature. The effect of temperature on halothane requirements in the toad was comparable to the effects of temperature in the goldfish and the dog. When halothane and methoxyflurane were carried in 100 per cent oxygen anesthetic requirements were higher than with 20 per cent oxygen. It made no difference whether nitrogen or helium was the third gas. Reserpinization of the toads abolished this effect of oxygen on anesthetic requirements, decreased anesthetic requirements, and reduced catecholamine concentrations in myocardium and brain. (Key words: Toad; Anesthetic requirements; General anesthetics; Oxygen.)

THE EFFECTS of anesthetics on sodium fluxes in the toad bladder [1] should be examined in the light of anesthetic requirements in the toad. In pilot studies it was noticed that with some anesthetic vapors less anesthetic was required when the vapor was carried in air rather than in oxygen. Accordingly, we designed a study to answer the following questions: a) What are the minimal anesthetic requirements in a cold-blooded animal like the amphibian at room temperature? b) Do temperature changes affect halothane anesthetic requirements in amphibians as they do in dogs [2] and goldfish [3]? c) Does the presence of higher nitrogen concentrations in the inhaled mixture decrease anesthetic requirements when compared wth mixtures of anesthetic and oxygen alone? Since nitrogen and helium were indistinguishable in their effects on minimal anesthetic requirements, we tested the hypothesis that oxygen stimulates the central nervous system and increases anesthetic requirements. This seemed plausible because Miller *et al.* have shown that reserpine decreases and catecholamines increase anesthetic requirements,[4] and because *in vivo* oxygen can cause vasoconstriction via sympathetic innervation.[5]

Methods

The toad, *Bufo marinus,* was used for the study. The toads were numbered and kept in the laboratory for months. Only healthy, acclimatized animals were used.

For the determination of anesthetic requirements for cyclopropane, nitrous oxide, ether, methoxyflurane, and halothane, we used minimum alveolar concentrations (MAC) as described by Eger *et al.*[6] The approximate MAC's were determined in pilot experiments. The trachea of each toad was intubated and its lungs ventilated with a volume larger and a rate slower than normal for toads. Mild hypocarbia was found not to affect MAC in the dog,[7] and we ascertained that moderate hyperventilation to a *p*H of about 7.6 had no effect upon MAC in five toads. This level of hyperventilation was used throughout the study. Most experiments were carried out at room temperature (20 to 22 C). Five determinations of MAC were done at 10 C and five at 30 C. At 10 and 20 C the rectal temperature of the toad was the same as the temperature in the environment. At 30 C the temperature of the toad rarely exceeded 27 C despite prolonged exposure. For determination of the anesthesia level a clamp was applied to a lower extremity for a few seconds. The force was slightly less than that necessary to produce visible injury to the skin but great enough so that additional pressure or stimulation did not

* Fellow.
† Professor.
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Address reprint requests to Dr. Andersen, Department of Anesthesiology, University Hospitals of Cleveland, 2065 Adelbert Road, Cleveland, Ohio 44106.

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TABLE 1. MAC Values for Five General Anesthetic Agents Carried in Oxygen in the Toad*

	Number of Toads	− Responses		+ Responses, End-expiratory Vol Per Cent	MAC (Vol Per Cent)
		Inspiratory Vol Per Cent	End-expiratory Vol Per Cent		
Cyclopropane	5	9.74 ± 0.82	9.54 ± 0.92	8.52 ± 0.33	9.0 ± 0.21
Nitrous oxide	5	85.4 ± 0.82	82.6 ± 0.2	81.8 ± 0.1	82.2 ± 0.1
Ether	5	2.19 ± 0.10	1.76 ± 0.12	1.51 ± 0.05	1.64 ± 0.04
Halothane	6	0.73 ± 0.01	0.70 ± 0.01	0.64 ± 0.01	0.67 ± 0.01
Methoxyflurane	5	0.35 ± 0.01	0.23 ± 0.01	0.21 ± 0.01	0.22 ± 0.01

* The concentrations associated with no response and with responses to a standard stimulus are shown

increase anesthetic requirements further. Inspired and end-expiratory anesthetic concentrations were determined by gas chromatography in an "F and M" flame ionization unit. The end-expiratory sample was collected through a catheter in the trachea with the aid of a slight circular pressure on the chest at the end of expiration. The toad was equilibrated with a gas or vapor tension well above the MAC value determined in pilot experiments. Small differences (table 1) between inspiratory and expiratory tensions were accepted, if constant.[8] Anesthesia was lightened and new equilibria attained in steps of about 1/10 of MAC. At each level the toad was tested for reaction to the standard stimulus. This was repeated until a muscle reaction was seen. Anesthesia was then deepened to the previous level, lightened and deepened again. When deepening and lightening of anesthesia in this way was associated with appropriate absence or presence of the reaction, and when the end-expiratory gas or vapor tension at each of the two levels was within ±5 per cent of the previous determination, MAC for that toad was defined as the mean value of the highest end-expiratory concentration associated with the reaction and the lowest concentration associated with no reaction. At least five toads were used for the assessment of MAC with each agent. Results were listed as mean ±1 standard error.

Minimal anesthetic requirement for thiopental sodium was related to the blood concentration of thiopental at the time of no reaction. The smallest intraperitoneal dose that would prevent reaction for five minutes was assessed as the mean (mg/kg) in ten toads from a dose–response curve. Doses of 10, 15, 20, or 25 mg/kg were injected three times each into toads randomly selected from the group. The times of no reaction were recorded, the dose–response curve drawn, and the dose associated with no reaction for five minutes (17.5 mg/kg) was determined from the curve. This dose was later injected into the same ten toads. Five toads were sacrificed as soon as the reaction disappeared during induction and five when the reaction reappeared during emergence. Immediately the toads were exsanguinated and the blood was cooled at 3 C and stored. Later the thiopental concentrations in the samples were determined according to the method of Brodie *et al.*[9] and minimal anesthetic requirements were ex-

TABLE 2. Blood Thiopental Concentrations in the Toad at the Times of No Reaction to a Standard Stimulus during Induction (Five Toads) and Beginning Reaction during Emergence (Five Toads)

	Blood Concentration (mg/100 ml)
Induction	3.96 ± 0.44
Emergence	1.69 ± 0.10
Mean	2.83 ± 0.45

TABLE 3. Approximate Minimum Anesthetic Requirements for Halothane (vol per cent in the Gas Phase) of the Goldfish,[3] Dog,[2] and Toad at Different Temperatures

	Degrees Centigrade			
	10-12	20-22	27-29	35-37
Goldfish	0.25	0.54	1.1	—
Toad	0.27	0.67	1.0	—
Dog	—	—	0.5	0.9

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TABLE 4. MAC Values in the Toad for Three General Anesthetic Agents Carried in 20 Per Cent Oxygen*

	Number of Toads	− Responses		+ Responses, End-expiratory Vol Per Cent	MAC (Vol Per Cent)
		Inspiratory Vol Per Cent	End-expiratory Vol Per Cent		
In 80 per cent nitrogen					
Ether	5	2.17 ± 0.01	1.81 ± 0.01	1.49 ± 0.05	1.64 ± 0.03
Halothane	6	0.59 ± 0.03	0.55 ± 0.02	0.48 ± 0.02	0.51 ± 0.02
Methoxyflurane	5	0.28 ± 0.04	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.01
In 80 per cent helium					
Halothane	6	0.56 ± 0.01	0.55 ± 0.01	0.50 ± 0.01	0.52 ± 0.01

* The concentrations associated with no response and with responses to a standard stimulus are shown.

pressed as the mean (mg/100 ml) of the ten samples ±1 standard error.

Forty-five toads were treated with crystalline reserpine (0.3, 1, or 6 mg/kg) dissolved in water and injected intraperitoneally. Twenty-four hours later, 15 of these toads and five untreated controls were sacrificed and the concentrations of epinephrine and norepinephrine in the cerebrum and the myocardium were determined according to the method of Anton and Sayre.[10] The rest of the reserpinized toads were used for the assessment of MAC for halothane.

Student's t tests were applied to the differences between groups to assess levels of significance.

Results

On the average it took ten minutes (cyclopropane, nitrous oxide) to 40 minutes (methoxyflurane) to achieve equilibrium between inhaled and exhaled concentrations of the anesthetic.

MAC values for the five gases and vapors in oxygen are shown in table 1. In addition, the highest end-expiratory concentrations associated with responses to the stimulus and the lowest end-expiratory and inspiratory concentrations with no responses are listed. The latter two indicate the difference between inspired and expired concentrations at equilibrium.

Table 2 shows minimal blood concentrations of thiopental needed for anesthesia. The differences between blood concentrations at comparable anesthetic levels during induction and emergence are included.

The effects of temperature changes on halothane requirements in the toad are shown in table 3. For comparison, the temperature effects in the dog[2] and the goldfish[3] are included.

For two of the anesthetics tested, halothane and methoxyflurane, MAC values in air were less than MAC values in oxygen ($P < 0.01$, table 4). With ether there was no difference between MAC in oxygen and MAC in air. MAC for halothane carried in a mixture of oxygen, 20 per cent, and He, 80 per cent, was the same as MAC for halothane in air (table 4).

Table 5 shows that increasing doses of reserpine reduced MAC for halothane in oxygen or air. Simultaneously the difference between MAC in oxygen and MAC in air gradually disappeared. After reserpine, 6 mg/kg, there was

TABLE 5. Halothane MAC Values in Oxygen and Air in Reserpinized Toads (Five Toads in Each Group)

Reserpine (mg/kg)	MAC (Vol Per Cent) in	
	Oxygen	Air
0.0	0.67 ± 0.01	0.51 ± 0.02
0.3	0.63 ± 0.01	0.50 ± 0.01
1.0	0.52 ± 0.02	0.46 ± 0.01
6.0	0.40 ± 0.01	0.40 ± 0.01

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TABLE 6. Concentrations of Epinephrine and Norepinephrine (Average Ratio = 10:1) in Reserpinized Toad Brain and Heart (Five Toads in Each Group; Means ± SE)

Reserpine (mg/kg)	Catecholamines (μg/g)	
	Brain	Heart
0.0	0.78 ± 0.08	6.59 ± 0.71
0.3	0.61 ± 0.11	3.28 ± 0.05*
1.0	0.57 ± 0.13	2.22 ± 0.52
6.0	0.43 ± 0.05*	2.24 ± 0.42

* Concentration significantly different from the concentration in untreated control, $P < 0.02$.

no difference between the two values. The degree of reserpinization was reflected by the catecholamine concentrations in brain and heart (table 6). Tissue catecholamines were reduced more in the heart than in the brain. Even in the fully-reserpinized animal a unit weight of heart contained three times more catecholamines than the same weight of brain in untreated animals. On the average, norepinephrine concentrations were 1/10 of epinephrine concentrations in both organs.

Discussion

Alveolar requirements necessary to achieve a certain level of anesthesia with the agents tested showed interrelationships similar to those described for the dog [6] and man.[8] Differences in the numerical values of MAC can be attributed to differences in temperature, species, and technique. The effect of temperature on halothane requirements in the toad was comparable to the temperature effects seen in the goldfish [3] and dog.[2] The differences between inspired and expired concentrations at equilibrium paralleled those described by Eger *et al.*[8]

Anesthetic requirements in the goldfish [3] and toad at 20 C appear to coincide with the requirements of the dog [2] and man [8] at body temperature. This may indicate that anesthetics are less potent in lower animals. Thiopental, which chemically is more active than most anesthetic gases and vapors, also was found to be equipotent at 20 C in the toad and 37 C in man.[9, 11-13]

The thiopental concentrations we found in blood may well have been in approximate equilibrium with the concentrations in the central nervous system. We believe the methodology justifies this statement, since: 1) the total dose was so small that it produced anesthesia for only about five minutes; 2) the uptake from the peritoneal cavity was so slow that anesthesia appeared 15 minutes after the time of injection; 3) the blood concentrations recorded were the mean values for induction and emergence.

We found it of particular interest that MAC values for methoxyflurane and halothane were less in air than in oxygen. Since halothane MAC was the same in 80 per cent nitrogen as in 80 per cent helium, we concluded that oxygen made the difference. Reserpinization of the toads abolished the effect of oxygen on MAC, decreased MAC, and reduced the catecholamine concentrations in myocardium and brain. Other observations also suggest that oxygen can influence the effects of anesthetics. Thus, Rawstron found that oxygen increased tolerance, decreased mortality, and prolonged induction with halothane.[14, 15] The mechanism for this oxygen effect, however, is not clear. Oxygen affected enzymes,[16] sympathetic activity,[6] and the cardiovascular system.[17] All of these are possible sites of interaction between oxygen and anesthetics. Reserpine and catecholamine depletion decreased MAC and decreased the oxygen effect on MAC. This supports but does not establish as fact our thesis that oxygen exerts its antianesthetic effect via catecholamines and the sympathetic nervous system.

Ether MAC was not affected by the oxygen concentration. Cyclopropane requirements may also be the same in oxygen as in air. At least, this was the case when the effect of cyclopropane, 9 per cent, and oxygen, 91 per cent, was compared with the effect of cyclopropane, 9 per cent, oxygen, 19 per cent, and nitrogen, 72 per cent (unpublished results). Perhaps ether and cyclopropane themselves stimulated the sympathetic system [1] and thereby overshadowed any excitatory effect that oxygen may have.

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References

1. Andersen NB, Shim CY: Sodium transport and anesthetic requirements in the toad. ANESTHESIOLOGY 34:338–343, 1971
2. Eger EI II, Saidman LJ, Brandstater B: Temperature dependence of halothane and cyclopropane anesthesia in dogs: Correlation with some theories of anesthetic action. ANESTHESIOLOGY 26:764–770, 1965
3. Cherkin A, Catchpool JF: Temperature dependence of anesthesia in goldfish. Science 144:1460–1562, 1964
4. Miller RD, Way WL, Eger EI II: The effects of alphamethyldopa, reserpine, guanethidine, and iproniazid on minimum alveolar anesthetic requirement (MAC). ANESTHESIOLOGY 29:1153–1158, 1968
5. Bauer CR, Gravenstein JS: Effect of oxygen on finger volume and heart rate in man. Anesth Analg 45:299–302, 1966
6. Eger EI II, Brandstater B, Saidman LJ, *et al.*: Equipotent alveolar concentrations of methoxyflurane, halothane, diethyl ether, fluroxene, cyclopropane, xenon and nitrous oxide in the dog. ANESTHESIOLOGY 26:771–777, 1965
7. Eger EI II, Saidman LJ, Brandstater B: Minimum alveolar anesthetic concentration: A standard of anesthetic potency. ANESTHESIOLOGY 26:756–763, 1965
8. Saidman LJ, Eger EI II, Munson ES, *et al.*: Minimum alveolar concentrations of methoxyflurane, halothane, ether and cyclopropane in man: Correlation with theories of anesthesia. ANESTHESIOLOGY 28:994–1002, 1967
9. Brodie BB, Mark LC, Papper EM, *et al.*: The fate of thiopental in man and a method for its estimation in biological material. J Pharmacol Exp Ther 98:85–96, 1950
10. Anton AH, Sayre DF: The distribution of dopamine and dopa in various animals and a method for their determination in diverse biological material. J Pharmacol 145:326–336, 1964
11. Mark LC, Perel JM, Brand L, *et al.*: Studies with thiohexital, an anesthetic barbiturate metabolized with unusual rapidity in man. ANESTHESIOLOGY 29:1159–1166, 1968
12. Kosaga Y, Takahaski T, Mark LC: Intravenous thiobarbiturate anesthesia for cesarean section. ANESTHESIOLOGY 31:489–506, 1969
13. Jailer JW, Boldbaum LR: Studies on the plasma concentration and tissue distribution of sodium pentothal (sodium ethyl (1-methylbutyl) thiobarbiturate). J Lab Clin Med 31:1344–1349, 1946
14. Rawstron RE: Oxgen and anemia: Their effects in mice on induction time and survival time with halothane anaesthesia. Brit J Anaesth 40:214–226, 1968
15. Rawstron RE: Oxygen and air. Further studies in mice with oxygen and air as the anaesthetic vehicles with halothane. Brit J Anaesth 41:475–480, 1969
16. Haugaard N: Cellular mechanisms of oxygen toxicity. Physiol Rev 48:311–373, 1968
17. Daly WJ, Bondurant S: Effects of oxygen breathing on the heart rate, blood pressure and cardiac index of normal men resting with reactive hyperemia and after atropine. Hemodynamic effects of oxygen breathing in normal man. J Clin Invest 41:126–132, 1962

Drugs

DPH AND ARRHYTHMIAS DPH (diphenylhydantoin) depressed automaticity, conduction and contractility in isolated perfused atria of rabbits and dogs. The depression resembled that seen after administration of quinidine. These findings are at variance with those of others, who have found improved conduction with DPH. The effects of DPH were markedly influenced by extracellular potassium—high concentrations enhance the effects, while low concentrations antagonized them. Increasing the concentrations of extracellular sodium also antagonized DPH effects. (*Katzung, B. G., and Jensen, R. A.: The Depressant Action of Diphenylhydantoin on Electrical and Mechanical Properties of Isolated Rabbit and Dog Atria: Dependence on Sodium and Potassium, Amer. Heart J. 80: 80 (July) 1970.*) ABSTRACTER'S COMMENT: It is fascinating to study the effects of various antiarrhythmic drugs on maximum following frequency, transmembrane action potentials, and conduction velocity in isolated perfused tissues—but difficult to apply this information in the treatment of arrhythmias in patients. Various ions, *p*H, local hypoxia, stretch, the automatic nervous system, and a host of other factors influence the response of the human heart to antiarrhythmic drugs. There ane also qualitative, as well as quantitative, differences in responses to low *vs.* high concentrations of drugs.

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