EFFECT OF ANESTHETICS ON THE STABILITY OF OXYHEMOGLOBIN S

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SUMMARY: Using a mechanical shake method, the effect of various anesthetics on the rate of precipitation of oxyhemoglobin S was studied. It was found that at clinically used concentrations halogenated anesthetics, such as halothane, fluroxene, and enflurane markedly accelerated the precipitation of oxyhemoglobin S, while diethyl ether prevented the precipitation. Further experiments are necessary to evaluate the clinical significance of the present findings.

INTRODUCTION

Patients with sickle cell disease are particularly vulnerable to the hazards of anesthesia and surgery since sickling of red cells occurs at low oxygen tensions. The resulting vascular stasis may in turn result in vascular occlusion, tissue hypoxia, and infarction (1). The heterozygous state of sickle cell disease known as sickle cell trait has generally been considered to be asymptomatic. However, it is known that widespread sickling and death can occur in persons with sickle cell trait under special circumstances such as during anesthesia when hypoxia has inadvertently occurred. McGary and Duncan (2) reported that five children with sickle cell trait died either during anesthesia or in the immediate post-operative period. Shapiro and Poe (3) reported that five out of fifteen patients with sickle cell disease who received

general anesthesia developed complications, whereas only one out of fourteen patients who received spinal anesthesia had difficulties. Although many factors including hypoxia and hypotension are involved in the development of complications, these results suggest that the clinical course may also be influenced by the type of anesthesia used.

Recently we developed a new method for investigating the stability of macromolecules, and found that the oxygenated form of sickle hemoglobin precipitates rapidly during mechanical shaking (4-6). In the present paper, we have investigated the effect of various anesthetics on the rate of precipitation of oxyhemoglobin S. The results clearly show that some anesthetics significantly accelerate the rate of precipitation of oxyhemoglobin S at clinically used concentrations under in vitro conditions. Although the clinical significance of the precipitation of oxyhemoglobin S must await further investigation, the present findings suggest that the selection of anesthesia for patients with sickle cell disease or sickle cell trait may be of importance.

MATERIALS AND METHODS

Hemoglobin solutions were prepared from fresh heparinized blood as described previously (4,5). Experiments were carried out either in 0.1 M Tris-HCl buffer (pH 8.0) or in a solution containing 100 mM KCl, 10 mM NaCl, 1 mM $MgCl_2$, 20 mM Tris-HCl and 5 mM phosphate buffer (pH 7.4). Essentially similar results were obtained in these two solutions (5). The following anesthetics were used: halothane (2-bromo-2-chloro-1:1:1-trifluorcethane (Ayerst Laboratories, New York, New York); fluroxene (trifluoroethyl vinyl ether) and enflurane (2-chloro-1,1,2-trifluoroethyl difluoromethyl ether) (both from Ohio Medical

Products, Airco, Madison, Wisconsin); cyclopropane (C_3H_6) and nitrous oxide (N_2O) (both from Chemetron Corporation, National Cylinder Gas Division, Chicago, Illinois); thiopental (Abbott Laboratories, North Chicago, Illinois) and ketamine (Park-Davis, Detroit, Michigan).

Shaking experiments were carried out in a 15 X 45 mm vial with a TCS shaker, Model P-1000 (P.O. Box 141, Southampton, Pennsylvania). Volatile anesthetics were bubbled with air into a 2 ml buffer solution for five minutes, 10 µl of stock hemoglobin solution (2.5 mM) was injected, gas was blown into the open space of the vial for thirty seconds and the vial was sealed. Concentrations of inhalational anesthetics (v/v gas) were determined by a Hewlett Packard (Palo Alto, California) Model 700 gas chromatograph. Cyclopropane and nitrous oxide were delivered from a standard anesthesia gas machine together with air and oxygen, respectively. After shaking, the vials were centrifuged at 3,600 r.p.m. for five minutes in order to separate precipitates, and the concentration of hemoglobin in the supernatant was determined with a TCS dual-wavelength spectrophotometer Model CL using wavelengths of 578 and 700 nm.

RESULTS AND DISCUSSION

As shown in Fig. 1, oxyhemoglobin S precipitates approximately tenfold faster than oxyhemoglobin A during mechanical shaking. Since the rate follows first order kinetics (6), the rate of precipitation can be expressed by the following equations:

$$Y = V + R, \qquad V = V_o \exp(-k \cdot t)$$

where Y is the total remaining hemoglobin in the supernatant, R the amount of stable hemoglobin, k the rate constant of precipitation, and V_o and V the amount of dissolved hemoglobin S

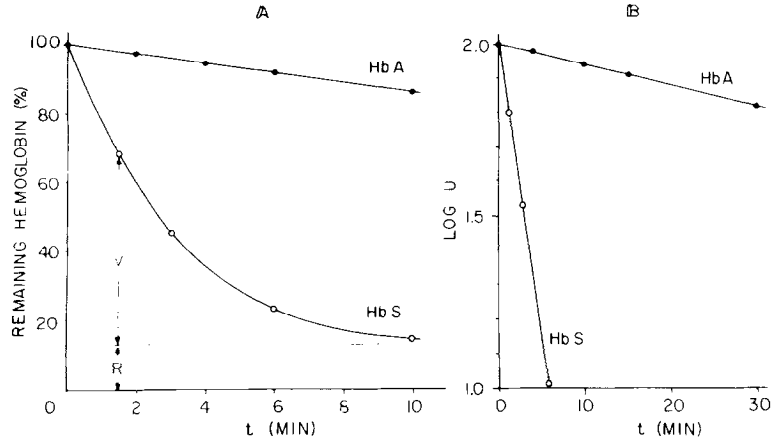

Fig. 1 Time course of precipitation of hemoglobin solution exposed to mechanical shaking. (A) Exponential decrease of oxyhemoglobin S (open circles) and A (closed circles). (B) Relation between log U and t (min.). U is the percentage of remaining oxyhemoglobin S ($U = V.100/V_O$) in the supernatant. Two ml of Tris-HCl buffer (pH 8.0) containing 12.5 uM hemoglobin were shaken for a certain length of time with a TCS shaker at 25° C, then centrifuged at 3,600 r.p.m. for five minutes. The concentration of remaining hemoglobin in the supernatant is measured spectrophotometrically.

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at time O and t (min.), respectively.

Hemolysates of sickle cell disease blood usually contain small concentrations of stable hemoglobin which does not precipitate easily by shaking (hemoglobin F). Therefore, the total concentration of this stable hemoglobin was expressed by R. When a proper value for R is found, a linear relationship is observed between the logarithm of percentage of remaining hemoglobin S ($U = V \cdot 100/V_O$) and the shaking time t (min.). The rate constant k can be obtained from the slope of the line (Fig. 1 (B)). A computer program was prepared to determine these parameters from experimental data (6).

The effect of various concentrations of halothane on the rate of precipitation of oxyhemoglobin S and A are compared in Fig. 2. The rate of precipitation of oxyhemoglobin S increased as the

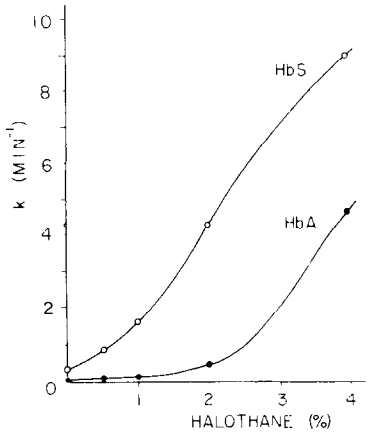
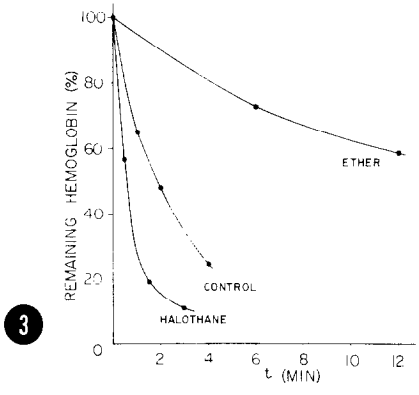


Fig. 2 Effect of different concentrations of halothane on the rate of precipitation of oxyhemoglobin S and A. Conditions are the same as in Fig. 1, except for the addition of different concentrations of halothane.

concentration of halothane was increased. On the contrary, halothane showed little effect on oxyhemoglobin A up to 1%, but then started to show an effect similar to that with oxyhemoglobin S. Fig. 3 compares the effects of halothane and diethyl ether on the rate of precipitation of oxyhemoglobin S. It is of interest that diethyl ether has a protective effect on oxyhemoglobin S, while halothane markedly accelerates the rate of precipitation. The effect of various concentrations of diethyl ether is shown in Fig. 4. The precipitation of oxyhemoglobin S is 84% inhibited in the presence of 1.55% (v/v gas) diethyl ether. Half inhibition took place at 0.3% concentration.

The effect of various anesthetics on the rate of precipitation of oxyhemoglobin S is summarized in Table 1, where the concentration of the inhalational anesthetics except for nitrous oxide was equal to the minimal alveolar anesthetic concentration (MAC, that concentration which will prevent 50% of patients from moving in response to a surgical skin incision, i.e., equipotent



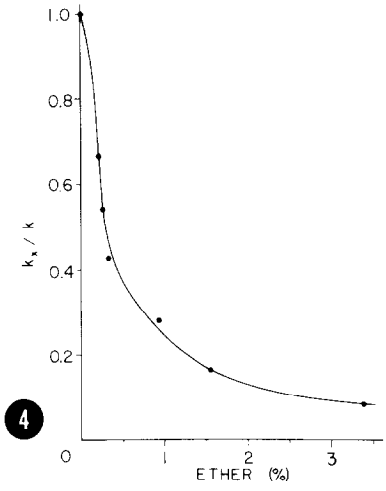


Fig. 3 Effect of halothane (0.63%) and diethyl ether (1.55%) on the rate of precipitation of oxyhemoglobin S. Conditions: 100 mM KCl, 10 mM NaCl, 1 mM $MgCl_2$, 20 mM Tris-HCl and 5 mM phosphate buffer (pH 7.4); 25° C.

Fig. 4 Effect of different concentrations of diethyl ether on the rate constant of precipitation of oxyhemoglobin S. The ordinate is expressed by the ratio of the rate constants (k_x/k), where k_x is the rate constant in the presence of certain amounts of diethyl ether. Conditions are the same as in Fig. 3.

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anesthetic concentration) in man (7). The concentration of intravenous anesthetics thiopental and ketamine is similar to the plasma concentration observed during clinical anesthesia. Thiopental, cyclopropane and nitrous oxide accelerated the precipitation to a lesser extent than halogenated anesthetics such as halothane, fluroxene and enflurane, while ketamine had almost no effect. Diethyl ether markedly inhibited precipitation.

The possibility of precipitation of hemoglobin S within the red cell as the first step leading to capillary stasis, deoxygenation and sickle cell formation has recently been raised (5). If certain anesthetics also enhance the precipitation of hemoglobin S within the intact red cell, then the choice of

TABLE I Effect of inhalational anesthetics (A) and intravenous anesthetics (B) on the rate constant of precipitation of oxyhemoglobin S. The results are shown by the ratio of k_x/k, where k_x and k are the rate constants in the presence and absence of anesthetics. These values are obtained by interpolation from dose response curves. Conditions: 100 mM KCl, 10 mM NaCl, 1 mM $MgCl_2$, 20 mM Tris-HCl and 5 mM phosphate buffer (pH 7.4); oxyhemoglobin, 12.5 μM; 25° C. Nitrous oxide is mixed with oxygen. Cyclopropane is mixed with air. All other inhalational anesthetics are vaporized in air. MAC = minimal alveolar anesthetic concentration (see text for explanation).

	Anesthetics	Concentration	MAC	(k_x/k)
	diethyl ether	1.92%	1	0.14
	nitrous oxide	80.0 %	0.8	1.7
(A)	cyclopropane	9.2 %	1	1.5
	halothane	0.74%	1	3.4
	fluroxene	3.4 %	1	8.7
	enflurane	1.68%	1	12.0
(B)	ketamine	2 μg/ml		0.96
	thiopental	20 μg/ml		2.4

particular anesthetic agents may affect the incidence of complications in patients with sickle cell disease or trait undergoing surgery. Further studies of the effects of anesthetics agents on hemoglobin S within the intact red cell under various clinical conditions are currently being performed.

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