The Effects of Volatile General Anesthetics on Adenosine Diphosphate-induced Platelet Aggregation

*Issaku Ueda, M.D.**

Platelet aggregation induced by ADP in canine platelet-rich plasma was inhibited by volatile anesthetics. The partial pressures at which platelet aggregation was inhibited 50 per cent correlated closely with the pressures used clinically: methoxyflurane, 3.5 torr; halothane, 7.5 torr; diethyl ether, 36 torr; cyclopropane, 120 torr. Nitrous oxide at 640 torr inhibited platelet aggregation 34 per cent. (Key words: Platelet aggregation; Volatile anesthetics.)

IN RECENT YEARS there has been a growing interest in the interaction between anesthetic agents and biological membranes. Clements and Wilson [1] demonstrated that a variety of general anesthetics decreased maximal surface tension of the phospholipid monolayer of the air–water interface, and correlated this phenomenon with anesthetic potencies. Seeman [2, 3] reported that a series of alcohols and steroids [2] and tranquilizers and local anesthetics [3] suppressed hypotonic hemolysis of erythrocytes. He advocated "membrane stabilization" of erythrocytes as a plausible model for the mechanism of anesthetic action.[2] We have reported that volatile anesthetics also protected erythrocytes from hypotonic hemolysis.[4]

Plasma separated from citrated whole blood by low centrifugal force contains a high concentration of platelets. Addition of adenosine diphosphate (ADP) to the platelet-rich plasma induces aggregation of platelets which can be measured quantitatively by a nephelometric method described by Born [5] and O'Brien.[6] Platelet aggregation apparently involves surface characteristics of the cell membrane.[7] Assuming that the primary site of action of anesthetics is at the cell membrane, I anticipated that anesthetics would modify platelet aggregation. The present study describes inhibitory effects of general anesthetics upon ADP-induced platelet aggregation.

Method

Canine blood was collected into siliconized bottles containing 4 per cent sodium citrate and 5 per cent glucose solution through teflon catheters inserted into dogs' jugular veins using local anesthesia. The final concentration of sodium citrate in the blood collected was 0.4 per cent. Some blood was collected from dogs paralyzed by intravenous injection of succinylcholine, 2 mg/kg, and given 70 per cent nitrous oxide and 30 per cent oxygen after tracheal intubation. Platelet-rich plasma was prepared by centrifuging citrated blood at 220 *g* for ten minutes. Siliconized glassware was used throughout.

Platelet aggregation was measured by the change of absorbancy at a wavelength of 600 nm with a Hitachi Perkin-Elmer 139 spectrophotometer at 25 C, and was recorded with a Brown-Honeywell Recorder. The cuvette compartment was modified so that two 25-gauge hypodermic needles could be introduced into the cuvettes. Anesthetics were bubbled through the hypodermic needle into a 1.0-cm light-path cuvette containing 2.0 ml of the platelet-rich plasma. After the anesthetic had bubbled for a minute, the reaction was started by adding 0.2 ml of ADP solution to the cuvette through the other needle. Increasing the equilibrium times of anesthetics to five minutes did not alter the inhibitory action. The plasma was stirred continuously by the bubbling of the anesthetics. Controls were

* Assistant Research Professor, Division of Anesthesiology, and Clinical Investigator, Veterans Administration Hospital.

Received from the Division of Anesthesiology, University of Utah College of Medicine, Salt Lake City, Utah 84112, and Veterans Administration Hospital, Salt Lake City, Utah 84113. Accepted for publication January 11, 1971.

obtained by bubbling compressed air into the plasma. To prevent the occurrence of foam during bubbling, needles were coated with silicone antifoam (Antifoam 60, General Electric).

Diethyl ether, halothane (Fluothane) and methoxyflurane (Penthrane) were vaporized in a Copper Kettle, and their concentrations were estimated from Kettle temperature and flow of diluent air. The accuracy of the calculated concentrations of the three anesthetics was confirmed with an Ohio gas chromatogram. The concentrations of cyclopropane and nitrous oxide were estimated from the ratios of flow of the anesthetics and air. Concentrations of the anesthetics were expressed as partial pressures (torr) in the gas phase.

Results

The rates of aggregation of platelets by ADP varied among plasma preparations. Those preparations which had absorbancies between 0.1 and 0.2 after the addition of ADP at a final concentration of 0.5 μM were used in this study. Twenty-three plasma preparations were used. Recordings of the rates of aggregation by various ADP concentrations in a representative preparation are superimposed and shown in figure 1. The aggregation occurred in a single phase. Biphasic responses have been reported in some preparations from cats,[8] guinea pigs,[9] and humans.[10, 11] The biphasic response, characterized by a secondary de-

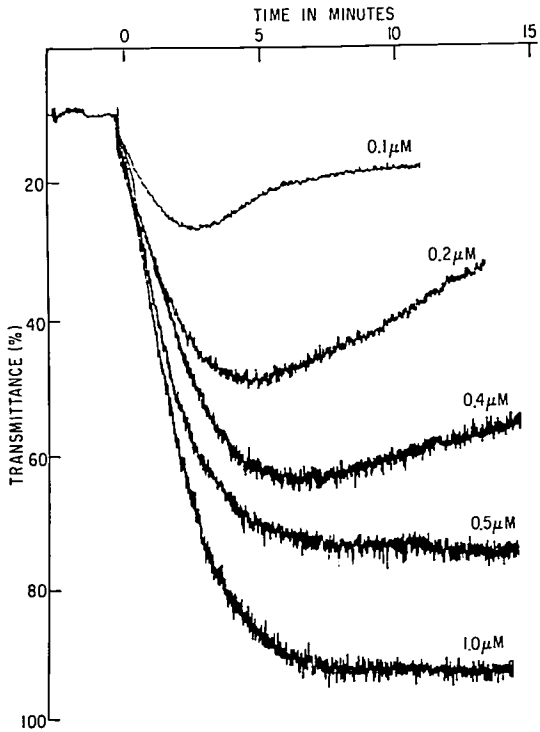

FIG. 1. Aggregation of platelets of canine plasma at various ADP concentrations. ADP was added to the platelet-rich plasma at zero time to final concentrations between 1.0 and 0.1 μM as indicated. At lower ADP concentrations the platelets disaggregated after reaching the peaks. Ordinate: Transmittance at 600 nm. Increased transmittance (or decreased absorbance) signifies a decreased number of particles in the light path. The number of particles in the light path is proportional to absorbance. Absorbance = log 100/transmittance (per cent) = 2 − log transmittance (per cent). Abscissa: Time in minutes.

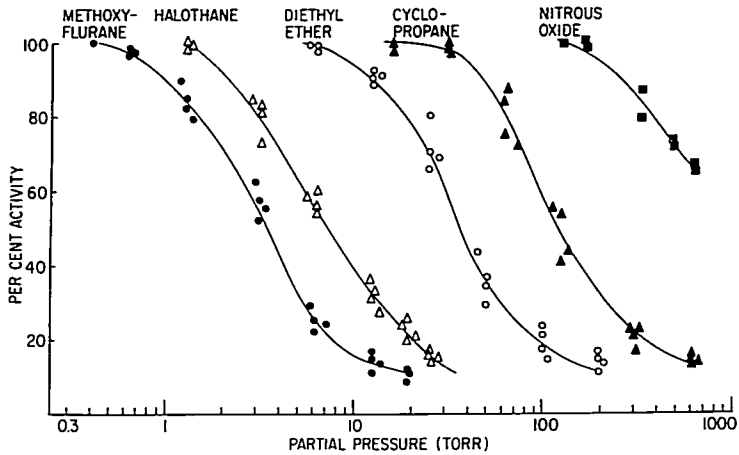

FIG. 2. The effects of anesthetics on platelet aggregation. Aggregation was induced by adding ADP to a final concentration of 0.5 μM. Each point is an average of triplicate observations. Ordinate: Per cent activity compared with the control values obtained by bubbling air. Abscissa: Estimated partial pressures of anesthetics in the gas phase in equilibrium with the platelet-rich plasma.

crease in absorbancy, was not observed in the present study. The cause of this discrepancy is not clear.

The effects of anesthetics on platelet aggregation are shown in figure 2. Aggregation was induced by adding ADP to a final concentration of 0.5 μM. All anesthetics inhibited ADP-induced aggregation. Partial pressures of the anesthetics which inhibited aggregation 50 per cent were: methoxyflurane, 3.5 torr; halothane, 7.5 torr; diethyl ether, 36 torr; cyclopropane, 120 torr. Nitrous oxide at 640 torr (an average atmospheric pressure at the location of this institution) inhibited aggregation 34 per cent.

Discussion

Platelet aggregation is receiving increasing attention as a model for drug interaction with cell membranes. Extensive reviews on platelet aggregation have appeared recently.[12, 13]

Depolarization of nerve cells in response to various stimuli is decreased by anesthetics. Numerous central depressants, local anesthetics, tranquilizers, and antihistaminics also prevent the shift of membrane potential of nerve cells. Shanes[14] classified these agents as "membrane stabilizers." Seeman[15] extended the concept of membrane stabilization from nerve cells to the erythrocytic membrane, and has shown that hypotonic hemolysis is preventable by a variety of the membrane stabilizers. Presumably, membrane stabilization by these agents is not limited to nerve cells, and analogies may be found in other cell membranes.

The present results suggest that anesthetics may change the surface characteristics of platelet cell membranes, thereby interfering with their cohesion. The inhibition of platelet aggregation observed may result from some nonspecific effects as organic solvents rather than from the anesthetic properties. Nevertheless, the tensions of anesthetics which inhibited platelet aggregation by 50 per cent showed a remarkable correlation with the tensions used clinically. Other membrane stabilizers, such as local anesthetics,[16] tranquilizers,[11] and anti-

histaminics,[11, 16-18] have also been reported to inhibit platelet aggregation. These results indicate that platelet aggregation may be a suitable model for analysis of the actions of centrally acting membrane stabilizers. The study of the binding of anesthetics to platelet membranes may contribute to the understanding of the mechanism of general anesthesia.

Fink and Kenny [19] reported that growth of monolayer cultures of mammalian cells was inhibited by anesthetics concomitant with decreases of oxygen consumption, and suggested that suppression of oxidative phosphorylation within mitochondria may be the cause of the inhibition of cell growth. In the presence of anesthetics, monolayer cells do not adhere to a glass plate (unpublished observation). Propagation of cells by this method depends on the ability of cells to adhere to glass plates.[20] It it possible that physicochemical changes in cell membranes effected by anesthetics may have suppressed the adhesive properties of the cell, as shown here with platelet aggregation, which in turn could inhibit growth of the monolayer culture. It has been reported that platelet aggregation [21] and cell growth *in vitro* [22] both were inhibited by cyclic adenosine 3',5'-monophosphate.

The author thanks Dr. W. A. Boyd for his help in preparing the manuscript.

References

1. Clements JA, Wilson KN: The affinity of narcotic agents for interfacial films. Proc Nat Acad Sci USA 48:1008–1014, 1962
2. Seeman P: Erythrocyte membrane stabilization by steroids and alcohols; a possible model for anesthesia. Biochem Pharmacol 15: 1632–1637, 1966
3. Seeman P: Erythrocyte membrane stabilization by local anesthetics and tranquilizers. Biochem Pharmacol 15:1753–1766, 1966
4. Okumura F, Koh J, Ueda I: Suppression of hypotonic hemolysis of washed human erythrocytes by general anesthetics. Jap J Anesth 17:186–187, 1968
5. Born GVR: Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature 194:927–929, 1962
6. O'Brien JR: Platelet aggregation. II. Some results from a new method of study. J Clin Path 15: 452–455, 1962
7. Aledort LM, Troup SB, Weed RI: Inhibition of sulfhydryl-dependent platelet functions by penetrating and non-penetrating analogues of parachloromercuribenzene. Blood 31: 471–497, 1968
8. Born GVR, Cross MJ: The aggregation of blood platelets. J Physiol 168:178–195, 1963
9. Constantine JW: Aggregation of guinea-pig platelets by adenosine diphosphate. Nature 210:162–164, 1966
10. MacMillan DC: Secondary clumping effect in human citrated platelet-rich plasma produced by adenosine diphosphate and adrenaline. Nature 211:140–144, 1966
11. Mills CB, Roberts CK: Membrane active drugs and the aggregation of human blood platelets. Nature 213:35–38, 1967
12. Michal F, Firkin BG: Physiological and pharmacological aspects of the platelet. Ann Rev Pharmacol 9:95–118, 1969
13. Mustard JF, Packham MA: Factors influencing platelet aggregation: Adhesion, release, and aggregation. Pharmacol Rev 22:97–187, 1970
14. Shanes AM: Electrochemical aspects of physiological and pharmacological action in excitable cells. Part I. The resting cell and its alteration by extrinsic factors. Pharmacol Rev 10:58–164, 1958
15. Seeman PM: Membrane stabilization by drugs: Tranquilizers, steroids, and anesthetics. Int Rev Neurobiol 9:145–221, 1966
16. O'Brien JR: The adhesiveness of native platelets and its prevention. J Clin Path 14:140–149, 1961
17. Herrmann RG, Frank JD: Effect of adenosine derivatives and anti-histaminics on platelet aggregation. Proc Soc Exp Biol Med 123: 654–660, 1966
18. Mitchell JRA, Sharp AA: Platelet clumping *in vitro*. Brit J Haematol 10:78–93, 1964
19. Fink BR, Kenny GE: Metabolic effects of volatile anesthetics in cell culture. ANESTHESIOLOGY 29:535–516, 1968
20. Cornell R: Cell–substrate adhesion during cell culture. An ultrastructural study. Exp Cell Res 58:289–295, 1969
21. Marquis NR, Vigdahl RL, Tavormina PA: Platelet aggregation. I. Regulation by cyclic AMP and prostaglandin E_1. Biochem Biophys Res Commun 36:965–972, 1969
22. Ryan WL, Heidrick ML: Inhibition of cell growth *in vitro* by adenosine 3',5'-monophosphate. Science 162:1484–1485, 1968