

# Effects of Volatile Anesthetics on Myocardial Oxidation-reduction Status Assessed by NADH Fluorometry

Igor Kissin, M.D., Ph.D.,\* D. F. Aultman, B.S.,† L. R. Smith, M.A.‡

In experiments on isolated rat heart perfused by the Langendorff method, the effect of halothane, isoflurane, enflurane, and diethyl ether on myocardial oxidation-reduction status was evaluated with reduced nicotinamide adenine dinucleotide (NADH) fluorometry. All inhaled anesthetics studied caused a dose-dependent increase in NADH fluorescence. Concentrations of anesthetics necessary to produce 10% of the maximal increase in NADH fluorescence caused by anoxia were 1.1% for halothane, 1.6% for isoflurane, 2.6% for enflurane, and 6.8% for diethyl ether (all concentrations are different from each other at  $P < 0.05$  level,  $n = 24$ ). These findings indicate that the order of potency with regard to the effect of the agent on NADH paralleled their potencies as general anesthetics. The deterioration in myocardial oxidation-reduction status probably is related to the ability of the anesthetic agents to inhibit the electron transport chain in mitochondria. (Key words: Anesthetics, intravenous; pentobarbital; thiopental. Anesthetics, volatile; diethyl ether; enflurane; halothane; isoflurane. Heart: contractility; redox state. Metabolism: mitochondria; NAD/NADH ratio.)

IT IS WELL KNOWN that barbiturates inhibit mitochondrial respiration at the level of nicotinamide adenine dinucleotide-reduced (NADH) dehydrogenase.<sup>1-3</sup> Amobarbital has been used as a selective tool to study the complexities of electron transport.<sup>4</sup> At the same time, it was found that barbiturate-induced decrease in myocardial contractility is related to inhibition of calcium release.<sup>5</sup> It has been reported that halothane also inhibits electron transport in the region of NADH dehydrogenase.<sup>6,7</sup> In experiments on the isolated heart, it was found that barbiturates<sup>8</sup> and halothane<sup>9</sup> increased myocardial NADH fluorescence. NADH fluorescence was suggested as an index for intracellular oxidation-reduction status.<sup>10-13</sup>

The aim of the present study was to determine whether different anesthetics affect the myocardial oxidation-reduction status in qualitatively similar ways. We have explored this question in a study of four inhaled anesthetics: isoflurane, enflurane, halothane, and diethyl ether. Two barbiturate anesthetics—thiopental and pentobarbital—also were investigated for comparison. NADH fluorometry was used to assess the oxidation-reduction state.

\* Professor, Anesthesiology.

† Senior Research Technician.

‡ Data Manager.

Received from the Department of Anesthesiology, University of Alabama in Birmingham Medical School, Birmingham, Alabama. Accepted for publication April 25, 1983.

Address reprint requests to Dr. Kissin: Department of Anesthesiology, The University of Alabama in Birmingham, University Station, Birmingham, Alabama 35194.

## Methods

Male Sprague-Dawley rats weighing 300–400 g were given heparin (1 mg/100 g) intraperitoneally one half hour prior to removal of the heart, and then the animals were killed by decapitation. The heart was excised, arrested in ice cold heparinized saline, and placed in a perfusion chamber (fig. 1). We used a Langendorff retrograde perfusion with nonrecirculating solution. The perfusate was Krebs-Henseleit bicarbonate buffer, pH 7.4 plus glucose (11 mM). Before use, it was filtered through a cellulose-acetate filter with 5  $\mu$ m pores (Millipore, Ltd.). The fluid was equilibrated with a 95% O<sub>2</sub> plus 5% CO<sub>2</sub> gas mixture (aortic O<sub>2</sub> partial pressure was over 600 mmHg). Perfusion was maintained by a Masterflex pump and the perfusion pressure held constant at 100 mmHg with a Starling-type resistor situated above the aortic cannula (compliance chamber). Temperature was controlled by warming the perfusate to 37°C, which was regulated thermostatically by a thermistor proximal to the aortic cannula and by separately controlling the perfusion chamber temperature at 37°C. It was shown by Opie *et al.*<sup>14</sup> that with this preparation, the pattern of glucose metabolism and oxygen tension of the effluent from the heart argues against hypoxia.

A latex balloon contoured to the left ventricle was introduced through the mitral valve and was secured to the mitral annulus with 7-0 silk sutures. The balloon was filled with saline through a micrometer syringe until end-diastolic pressure stabilized at 4–5 mmHg. It was then assumed that the left ventricle was contracting isovolumetrically. The balloon was connected through a 20-cm long, stiff polyvinylchloride tube to a Gould-Statham P23 pressure transducer. Left ventricular dP/dt max was recorded with a Grass 7D<sup>®</sup> polygraph. To prevent accumulation of perfusion fluid between the latex balloon and the walls of the left ventricle (due to Thebesian drainage), the ventricle was vented through the apex. This was done before the placement of the latex balloon, with the use of a 1.2-cm long polyethylene catheter (0.86 mm I.D., 1.27 mm O.D.; one end of the catheter was widened slightly by heating). The catheter was inserted through the ventricular wall with a 22-ga needle used as an introducer (the catheter was placed over the needle and directed through the mitral valve). After insertion, the needle was removed and the catheter was left in place with the widened end inside. A bipolar electrode was attached to the right atrium and the heart paced at a

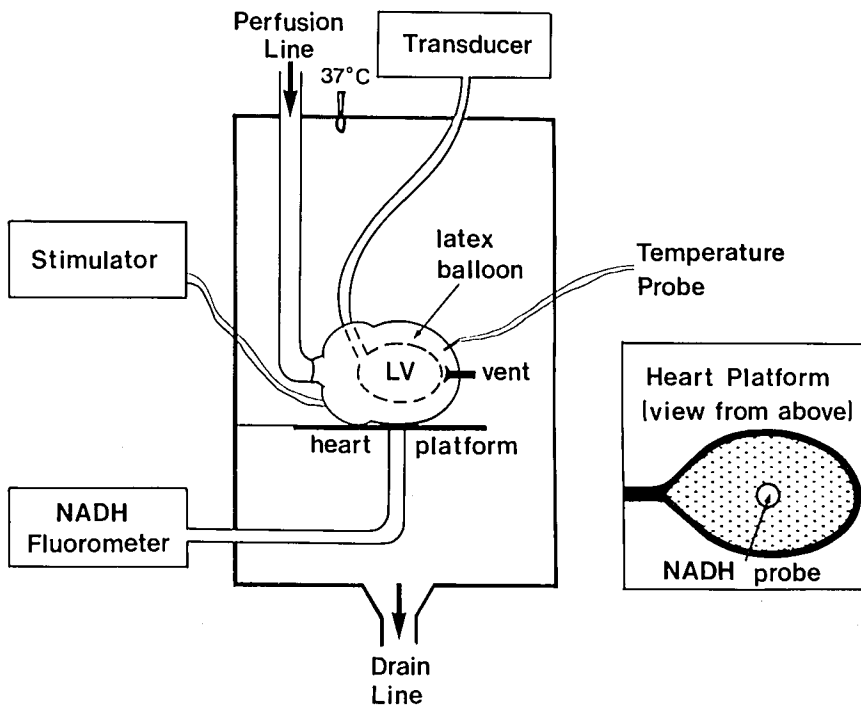


FIG. 1. Schematic illustration of the experimental setup.

frequency of 300/min with rectangular impulses (2.5 ms; voltage 25% above threshold) provided by a Grass S88<sup>®</sup> stimulator.

Chance and associates<sup>10</sup> devised a technique for assessing the tissue oxidation-reduction state based on the fluorescent characteristics of the pyridine nucleotide-reduced pyridine nucleotide (NAD-NADH) system, the initial member of the respiratory chain. NADH fluorometry permits the continuous measurement of intracellular redox state without damage to myocardial tissue. Pyridine nucleotide changes were monitored continuously with a DC fluorometer/reflectometer from the epicardial surface of the left ventricle. To accomplish this, the heart was placed horizontally on the platform with the light guide end of the fluorometer (3-mm diameter) in its center (fig. 1). The light guide supplied 366 nm excitation light to the surface of the heart through one group of fibers and transmitted fluorescence emission (460 nm) back through a second group of fibers for processing. Extraneous light was shielded from the heart. The output from the fluorometer was displayed on the Grass polygraph. The relative NADH fluorescence intensity was expressed as a percentage of the difference between the initial fluorescence signal (obtained at the end of equilibration period) and the maximal fluorescence signal obtained after a 3-min anoxic period.

Following a 45-min equilibration period, the heart was exposed to an anesthetic. The concentration of the anesthetic in the perfusion fluid progressively was increased in four steps each over a 10-min period. These steps had been predetermined to give myocardial depression ( $dP/dt$ )

between approximately 20% and 80% of control values. Maximal changes in NADH fluorescence and  $dP/dt$  were used for calculations. After the fourth step, the heart was perfused with fluid without an anesthetic for an additional 20 min. During this period, the variables returned to the preanesthetic levels. Following this period, the perfusion was stopped for three min, and the maximal fluorescence signal, caused by anoxia, was obtained for the calculations. Volatile anesthetics were administered into the fluid of the perfusion reservoir by means of calibrated vaporizers or a copper kettle using the  $O_2/CO_2$  mixture (at 3 l/min) as the carrier gas. The anesthetic-containing mixture was bubbled through the solution for 20 min before application. The change in an agent concentration was achieved by changing the perfusion reservoir. Anesthetic concentrations in the solutions perfused over the myocardium were checked with gas chromatography.<sup>15</sup> Barbiturates (thiopental or pentobarbital) were mixed in 500 ml perfusate for each of the four concentrations.

A group of six experiments were performed with each of the six agents used in the study. The anesthetic concentration required to cause 10% of maximal increase (caused by anoxia) in NADH fluorescence was calculated from derived linear regression in each experiment. The method of least squares was used to determine the log-concentration effect relationship. The same log-concentration effect curves were used to calculate NADH changes at the equianesthetic concentrations of the volatile anesthetics. Agent concentrations that block movement response to nociceptive stimuli were taken as the basis

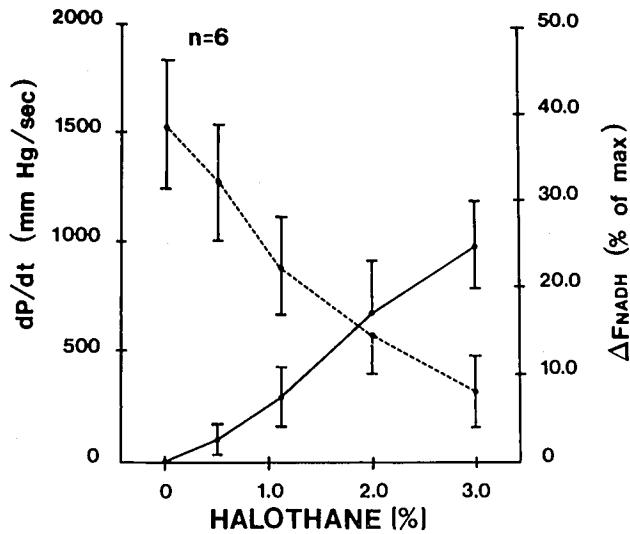


FIG. 2. Effect of halothane on myocardial NADH fluorescence. Points represent mean  $\pm$  SEM, the solid line is NADH, the interrupted line is dP/dt. The relative NADH fluorescence intensity was expressed as a percentage of the maximal fluorescence signal obtained after a 3-min anoxic period.

for the calculations. Because we could not find rat MAC data in literature for all of the studied agents, "inspired" ED<sub>50</sub> values<sup>16,17</sup> were used for calculations (which give almost the same anesthetic potency ratios as MAC values). ED<sub>50</sub> values were 1.1% for halothane, 1.7% for isoflurane, 2.0% for enflurane, 3.1% for diethyl ether. The means for each group of experiments were calculated from the individual values.

For correlation of the effects of anesthetics on NADH fluorescence and dP/dt, the NADH-dP/dt data were fitted with a cubic spline curve. The NADH fluorescence

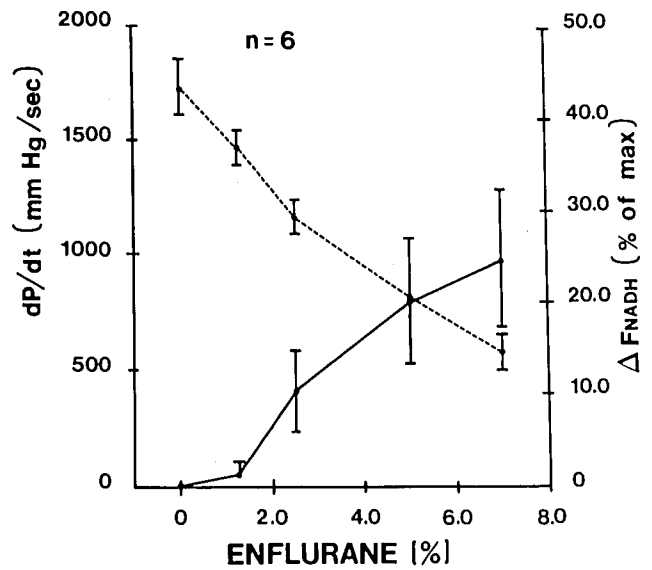


FIG. 4. Effect of enflurane on myocardial NADH fluorescence.

values were calculated from the derived curve at agent concentrations that decreased dP/dt by 10, 20, 30, 40, 50, and 60% in each experiment. The mean NADH values were plotted against mean dP/dt values for each anesthetic. To determine the significant difference between means for different anesthetics, Duncan multiple-range test was used.

### Results

All studied volatile anesthetics caused an increase in NADH fluorescence. In pharmacologic concentrations, each of the anesthetics produced a dose-related effect (figs. 2-5). Effects of volatile anesthetics were comparable

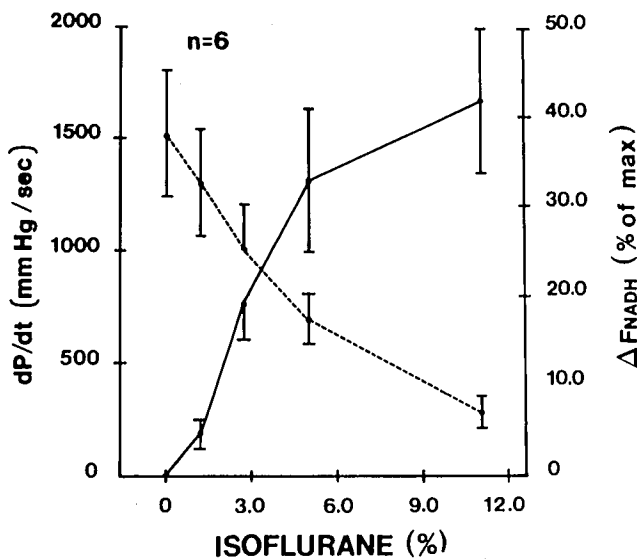


FIG. 3. Effect of isoflurane on myocardial NADH fluorescence.

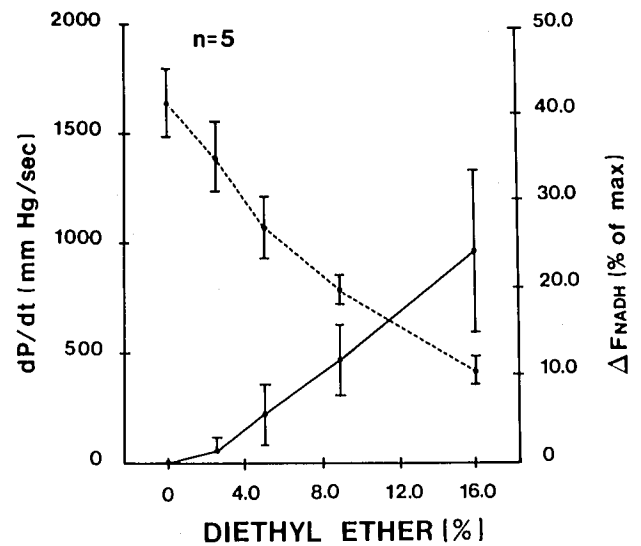


FIG. 5. Effect of diethyl ether on myocardial NADH fluorescence.

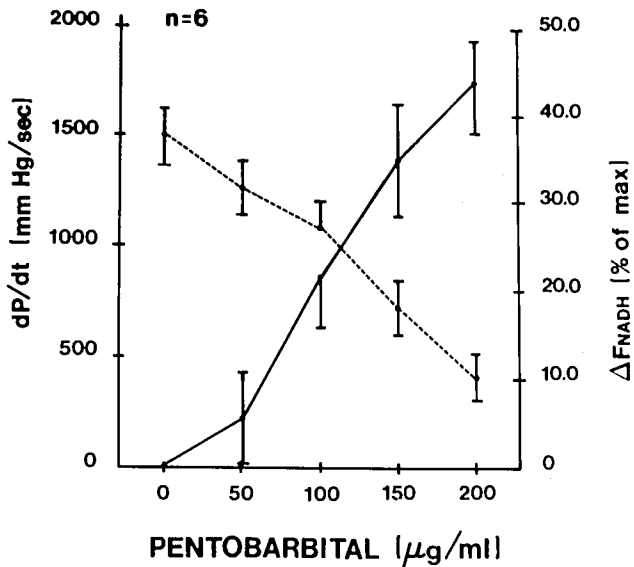


FIG. 6. Effect of pentobarbital on myocardial NADH fluorescence.

to those of barbiturate agents (figs. 6 and 7). Concentrations of anesthetics necessary to produce a 10% of maximal increase in NADH fluorescence caused by anoxia were 1.1% for halothane, 1.6% for isoflurane, 2.6% for enflurane, and 6.8% for diethyl ether. Differences between these numbers are statistically significant ( $P < 0.05$ ).

Figure 8 represents NADH effects of the volatile agents at equianesthetic concentrations. It is possible to see that when the inhaled anesthetics are viewed in terms of the degree of NADH fluorescence increase produced by equianesthetic concentrations, their NADH effects are quantitatively different. Isoflurane produced the most

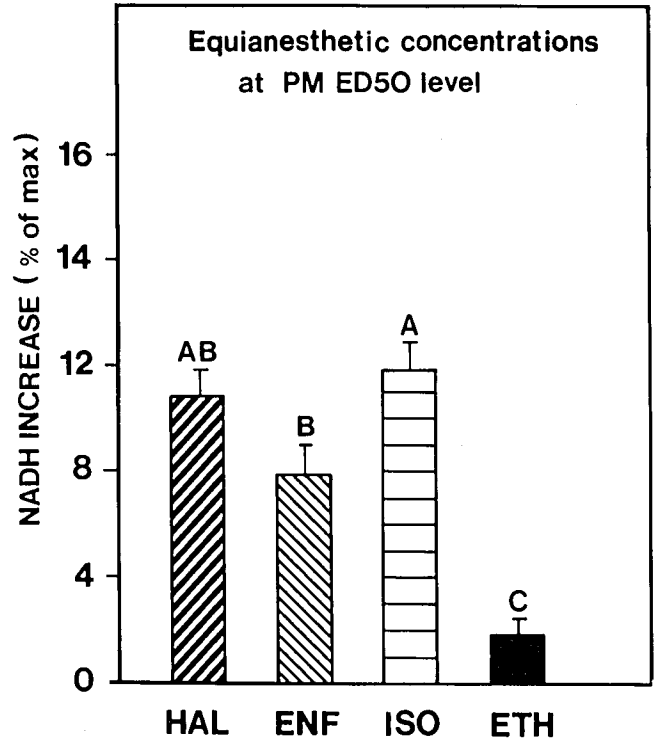


FIG. 8. Effect of four volatile anesthetics on myocardial NADH fluorescence at equianesthetic concentrations. The NADH fluorescence value in each experiment was calculated from derived linear regression at an anesthetic concentration corresponding to the inspired  $ED_{50}$  value that prevents purposeful movement in response to pain stimulation (PM  $ED_{50}$  values<sup>16,17</sup> for calculations were 1.1% for halothane, 1.7% for isoflurane, 2.0% for enflurane, and 3.1% for diethyl ether). Columns represent mean  $\pm$  SEM calculated from individual values for each group of experiments. Letters above the columns represent the significance of the difference as determined by Duncan's multiple-range test. Columns with the same letter are not significantly different ( $P < 0.05$ ). Explanations in the text.

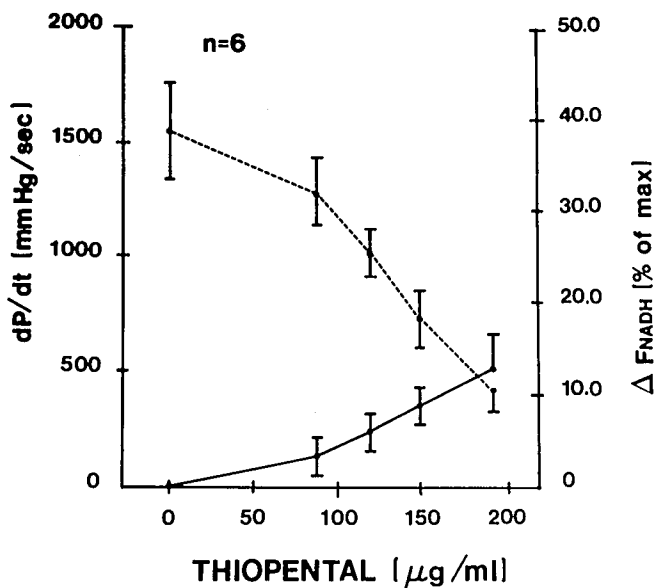


FIG. 7. Effect of thiopental on myocardial NADH fluorescence.

pronounced changes; diethyl ether produced the least. The effect of halothane was very similar to that of isoflurane, while the effect of enflurane was significantly less pronounced than that of isoflurane.

Table 1 represents a comparison of the effects of the anesthetics on NADH fluorescence at different levels of contractility depression (decrease in dP/dt by 10, 20, 30, 40, 50, and 60%). The anesthetics were ranked according to the degree of the NADH effect. Isoflurane and pentobarbital had the highest rank for NADH increase at all levels of contractility depression. Enflurane was ranked as third in all but the 10% level of contractility depression. Halothane, diethyl ether and thiopental clustered as lower ranks. Figure 9 shows the percentage of increase in NADH fluorescence induced by the anesthetics at concentrations causing 50% decrease in dP/dt. It is possible to see from this figure that various anesthetics cause different degrees of increase in NADH fluorescence, despite the same level of depression of myocardial contractility.

**Discussion**

The anesthetic-induced increase in NADH fluorescence obtained in our experiments agrees with the reported data showing that halothane in relatively small concentrations inhibits the oxidation of NAD-linked substrates in heart mitochondria.<sup>6</sup> It also agrees with the results of experiments that were performed in the isolated perfused rat liver and demonstrated the decrease in calculated NAD/NADH ratio under the influence of halothane.<sup>18</sup> Since barbiturates and halothane inhibit the electron transfer chain,<sup>2,4,6,7</sup> they should cause an accumulation of reduced form of pyridine nucleotide (NADH). Therefore, NADH fluorescence would appear to be an adequate index for the investigation of the effect of halothane and other anesthetic agents on the myocardial oxidation-reduction state.

We have found that the order of potency with regard to the effect of inhaled anesthetics on NADH fluorescence paralleled their potencies as general anesthetics. The order of potency gives a relatively low degree of precision in the measurement of correlation between the two actions. When we compared (fig. 8) the NADH effect of the volatile agents at equianesthetic concentrations, we found that their NADH effects are quantitatively different. Isoflurane produced the most pronounced changes, while diethyl ether produced the least.

If an energy supply defect, due to inhibition of mitochondrial respiration, is involved in the effect of volatile anesthetics on the myocardium and it is not offset by decreased energy demand, then the myocardial ATP level may be decreased. In 1975, Merin<sup>19</sup> stated that there had been no adequate documentation of a decrease in ATP in myocardium for pharmacologic doses of anesthetics. Neither a decrease nor an increase in the ATP myocardial level has been demonstrated with halothane since then.<sup>20-22</sup> One possible explanation for this is the intracellular compartmentation of ATP and creatine phosphate (total tissue ATP level would not be affected appreciably by even a significant change in one of the many compartments).<sup>23,24</sup> At the same time, it was reported that increasing concentrations of halothane decreased the rate of ATP turnover.<sup>20</sup> The authors regarded this fact as evidence that suggests that halothane blocks electron transport at the NADH-coenzyme Q reductase level.

It has been suggested<sup>7,25</sup> that various anesthetic agent actions, including inotropic effects, may result from the depression of energy production. Therefore, it was of interest to compare the negative inotropic and NADH effects of the anesthetics. While correlating effects of anesthetics on NADH fluorescence and dP/dt, it should be taken into account that NADH fluorescence reflects the NAD/NADH ratio in the epicardial layers of myocardium

TABLE 1. Ranking of Anesthetics According to NADH Fluorescence Effect at Different Levels of Contractility Depression

10%*	20%*	30%*	40%*	50%*	60%*
I†	P	P	P	P	I†
P†	I	I	I	I	P†
T†	E†	E	E	E	E
H†	T†	T†	D†	D†	H†
E†	H†	H†	T†	H†	D†
D†	D†	D†	H†	T†	T

For each level of contractility depression, the anesthetics were ranked in decreasing NADH response.

I = isoflurane; P = pentobarbital; E = enflurane; H = halothane; D = diethyl ether; T = thiopental.

\* Percentage of decrease in dP/dt.

† Mean NADH increases of the adjacent anesthetics are not significantly different at the P = 0.05 level (Duncan's multiple-range test).

and dP/dt represents the overall contractility of the left ventricle. Although there is no direct proof that the NAD/NADH ratio from the superficial layers is truly representative of the deeper lying cells, there is evidence for such an assumption. First, when NADH fluorescence photographs of the myocardium were taken in successive, measured layers (three dimensional display of NADH fluorescence), the deep layers of myocardium gave the same type of changes in NADH fluorescence as the superficial layer.<sup>26</sup> Second, changes in NADH fluorescence obtained from epicardial and endocardial surfaces of the heart were very similar,<sup>9</sup> despite the fact that endocardial and epicardial layers of the myocardium usually show the

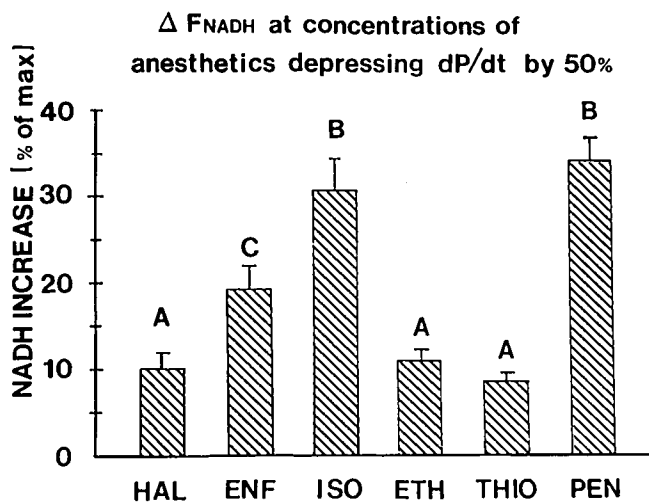


FIG. 9. Effect of anesthetics on myocardial NADH fluorescence at 50% contractility depression. For each experiment, the NADH-dP/dt data were fitted with a cubic spline curve. The NADH fluorescence value was calculated from the derived curve at an agent concentration that decreased dP/dt by 50%. Columns represent mean  $\pm$  SEM calculated from individual values for each group of experiments. Columns with the same letter are not significantly different ( $P < 0.05$ ) as determined using Duncan's multiple-range test.

maximal difference with regard to the oxygen demand-supply balance.

It is possible to see from table 1 and figure 9 that various anesthetics cause different degrees of increase in NADH fluorescence, despite the same level of depression in myocardial contractility. This fact may be regarded as additional evidence for the suggestion<sup>19</sup> that depression of myocardial contractility under the influence of volatile anesthetics is not an effect secondary to inhibition of mitochondrial electron transport.

Thus, all studied volatile anesthetics cause an increase in NADH fluorescence that reflects deterioration in myocardial oxidation-reduction state. With concentrations of anesthetics equivalent to their therapeutic doses, this effect probably does not exceed 15–20% of maximal deterioration in oxidation-reduction state caused by anoxia. It is accepted commonly that the mechanism of action of volatile anesthetics is due to their physicochemical properties and, therefore, not very specific. In the spectrum of anesthetic agent actions, there are many adverse effects and the effect on myocardial oxidation-reduction state is one of them. It is possible to speculate that the relatively small deterioration in myocardial oxidation-reduction state caused by anesthetics may be an important factor in myocardial ischemia. However, it was found with halothane, that in myocardial ischemia, there was no increase in NADH fluorescence after anesthetic administration.<sup>9</sup>

### References

1. Quastel JH, Wheatly AHM: Narcosis and oxidations of the brain. *Proc R Soc Lond [Biol]* 112:60–79, 1932
2. Chance B, Cohen P, Jobsis F, Schoener B: Intracellular oxidation-reduction states in vivo. *Science* 137:499–508, 1962
3. Chance B, Hollinger G: Inhibition of electron and energy transfer in mitochondria. *J Biol Chem* 278:418–431, 1963
4. Chance B, Ernster L, Garland PB, Lee CP, Light PA, Ohnishi T, Regal CI, Wong D: Flavoproteins of the mitochondrial respiratory chain. *Proc Natl Acad Sci USA* 57:1498–1505, 1967
5. Nayler WG, Szeto J: Effect of sodium pentobarbital on calcium in mammalian heart muscle. *Am J Physiol* 222:339–344, 1972
6. Harris RA, Munroe J, Farmer B, Kim KC, Jenkins P: Action of halothane upon mitochondrial respiration. *Arch Biochem Biophys* 142:435–444, 1971
7. Berman MC, Kewley CF, Kench JE: Contribution of inhibition of NADH-dehydrogenase to cardiotoxic effects of halothane. *J Mol Cell Cardiol* 6:39–47, 1974
8. Weiss JP, Barlow CH, Chance B: Pentobarbital-induced reduction of pyridine nucleotide measured by surface fluorometry in perfused rat heart. *Biochem Pharmacol* 27:1510–1511, 1978
9. Kissin I, Thomson CT, Smith LR: Effect of halothane on contractile function of ischemic myocardium. *Cardiovasc Pharmacol* 5:438–442, 1983
10. Chance B, Mayevsky A, Goodwin C, Mela L: Factors in oxygen delivery to tissue. *Microvasc Res* 8:276–282, 1974
11. Franke H, Barlow CH, Chance B: Oxygen delivery in perfused rat kidney: NADH fluorescence and renal functional state. *Am J Physiol* 231:1082–1088, 1976
12. Pearce FJ, Forster J, DeLeeuw G, Williamson JR, Tutwiler GF: Inhibition of fatty acid oxidation in normal and hypoxic perfused rat hearts by 2-tetradecylglycidic acid. *J Mol Cell Cardiol* 11:893–915, 1979
13. Wetstein L, Simson MB, Feldman PD, Harken AH: Pharmacologic modification of myocardial ischemia. *Circulation* 66:548–554, 1982
14. Opie LH, Shipp JC, Evans JR, Leboeuf B: Metabolism of glucose-U-<sup>14</sup>C in perfused rat heart. *Am J Physiol* 203:838–843, 1962
15. 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
16. Kissin I, Morgan PL, Smith LR: Isoflurane vs. halothane: Safety margins. *ANESTHESIOLOGY* 57:A370, 1982
17. Kissin I, Morgan PL: Heart rate response to nociceptive stimulation as an index of anesthetic potency for enflurane. *ANESTHESIOLOGY* 58:109–110, 1983
18. Biebuyck JF, Lund P, Krebs HA: The effects of halothane on glycolysis and biosynthetic processes of the isolated perfused rat liver. *Biochem J* 128:711–720, 1972
19. Merin RG: Subcellular mechanisms for the negative inotropic effect of inhalation anesthetics, *Molecular Mechanisms of Anesthesia*. Progress in Anesthesiology, vol 1. Edited by Fink BR. New York, Raven Press, 1975, p 607
20. Strong LJ, Hartzell CR, McCarl RL: Halothane and the beating response and ATP turnover rate of heart cells in tissue culture. *ANESTHESIOLOGY* 42:123–132, 1975
21. Merin RG, Verdouw PD, deJong JW: Dose-dependent depression of cardiac function and metabolism by halothane in swine (*Sus scrofa*). *ANESTHESIOLOGY* 46:417–423, 1977
22. Peyton R, Christian C, Fagraens L, Van Trigt P, Spray T, Pellom G, Pasque M, Wechsler A: Halothane and myocardial protection. *ANESTHESIOLOGY* 57:A9, 1982
23. Gudbjarnason S, Mathes P, Ravens KG: Functional compartmentation of ATP and creatine phosphate in heart muscle. *J Mol Cell Cardiol* 1:325–339, 1970
24. Nunnally RL, Hollis DP: Adenosine triphosphate compartmentation in living hearts: A phosphorus nuclear magnetic resonance saturation transfer study. *Biochemistry* 18:3642–3647, 1979
25. Quastel JH: Effects of drugs on metabolism of the brain in vitro. *Br Med Bull* 21:49–56, 1965
26. Harken AH, Barlow CH, Harden WR, Chance B: Two and three dimensional display of myocardial ischemic "border zone" in dogs. *Am J Cardiol* 42:954–959, 1978