

# Responses to Fasting and Lipid Infusion of Epinephrine-induced Arrhythmias during Halothane Anesthesia

David J. Miletich, Ph.D.,\* Ronald F. Albrecht, M.D.,† Chresteen Seals, B.S.‡

Sensitivities to epinephrine in fasted and non-fasted rats anesthetized with halothane or pentobarbital were determined. The arrhythmic threshold for epinephrine, *i.e.*, the dose which upon injection produced four or more premature ventricular contractions, was significantly lower for 12-hour ( $5.5 \pm 3.2 \mu\text{g}/\text{kg}$ ), 24-hour ( $2.2 \pm 1.3 \mu\text{g}/\text{kg}$ ) and 48-hour ( $2.25 \pm 0.6 \mu\text{g}/\text{kg}$ ) fasted rats than for non-fasted rats ( $10.9 \pm 3.4 \mu\text{g}/\text{kg}$ ) at the same dose of halothane (2.0 per cent). Fasting had no effect on the arrhythmic threshold for epinephrine in rats anesthetized with pentobarbital (35 mg/kg). Infusion of a 10 per cent fatty acid emulsion caused a significant lowering of the arrhythmic threshold for epinephrine of fed rats, while infusion of acetoacetic acid had no effect on the arrhythmic threshold. It is concluded that the combination of fasting and halothane anesthesia renders the heart more sensitive to epinephrine than halothane anesthesia alone. (Key words: Anesthetics, volatile, halothane; Anesthetics, intravenous, pentobarbital; Heart, arrhythmias; Heart, epinephrine; Heart, metabolism.)

INCREASED IRRITABILITY of the heart in response to catecholamines during halothane anesthesia, resulting in arrhythmia, is a well-documented phenomenon.<sup>1</sup> Increased blood pressure,<sup>2</sup> rapid heart rate,<sup>3</sup> increased blood potassium,<sup>4</sup> and conduction defects<sup>5</sup> have all been demonstrated as predisposing factors that facilitate arrhythmias during anesthesia. To date, no consideration has been given to the possibility that particular states of myocardial metabolism under certain conditions may contribute to the production of arrhythmias. In light of reports that implicate increased blood free fatty acids as a factor contributing to arrhythmias following myocardial infarction,<sup>6,7</sup> it was the intent of this study to evaluate what effects, if any, fasting might have on the occurrence of arrhythmias during halothane anesthesia. For this purpose epinephrine arrhythmogenicity was determined in fasted and fed rats exposed to halothane or pentobarbital anesthesia.

## Methods

Common white, male rats weighing 200–250 g were used throughout the study. Rats were housed in

groups of four and maintained on wood-chip bedding. All rats were fed Purina Chow Lab Blox (65 per cent carbohydrate, 25 per cent protein, 4 per cent fat, 4 per cent crude fiber).

The effects of test anesthetics and fasting on epinephrine-induced arrhythmia were evaluated by measuring changes in the epinephrine arrhythmic threshold as described by Zahed *et al.*<sup>8</sup> The epinephrine arrhythmic threshold is the dose of epinephrine which upon intravenous injection causes four or more continuous or intermittent premature ventricular contractions within 15 sec. It is derived by mathematical interpolation from the dose of epinephrine that produces at least four premature ventricular contractions and a lower dose that does not produce arrhythmia. The lower dose was purposely adjusted so that it was below the arrhythmia-producing dose by  $0.5 \mu\text{g}/\text{kg}$ . For example, if  $10 \mu\text{g}/\text{kg}$  epinephrine produced four or more premature ventricular contractions while  $9.5 \mu\text{g}/\text{kg}$  did not, the arrhythmic threshold would be taken to be  $9.75 \mu\text{g}/\text{kg}$ .

Animals were surgically prepared for study following induction of anesthesia with the test anesthetic, *i.e.*, either halothane, 1 per cent, in oxygen, or sodium pentobarbital, 35 mg/kg. After tracheostomy the rats were mechanically ventilated with a small-animal respirator set to deliver a tidal volume of 3.5 ml at 40–50 breaths/min. Respiratory rate was adjusted until an arterial blood carbon dioxide partial pressure of 30–40 torr was achieved. Catheters were inserted into a carotid artery for monitoring blood pressure by transducer and into a subclavian vein, which was attached to a fixed-needle Hamilton Standard gas chromatograph syringe of 100- $\mu\text{l}$  volume. This apparatus was used for the administration of test doses of epinephrine. The injection procedure was controlled by an automatic repeating dispenser that delivered exactly  $2 \mu\text{l}$ . A standard epinephrine solution ( $0.25 \mu\text{g}/\mu\text{l}$  saline solution) was made up in advance and several dozen 2- $\mu\text{l}$  volumes frozen for subsequent use each day.

Following completion of the surgical preparation, standard lead II of the electrocardiogram was obtained by placing a needle electrode in each limb. Before administration of epinephrine the concentration of halothane was increased to 2 per cent in oxygen and held steady for 10 minutes. When sodium pentobarbital was given, a maintenance dose of 20 mg/kg

\* Assistant Professor of Anesthesiology, University of Chicago at Michael Reese Hospital and Medical Center.

† Professor of Anesthesiology, University of Chicago; Chairman at Michael Reese Hospital and Medical Center.

‡ Research Assistant.

Received from the Michael Reese Hospital and Medical Center, Chicago, Illinois 60616. Accepted for publication October 10, 1977.

Address reprint requests to Dr. Miletich.

TABLE 1. Arrhythmic Threshold Doses of Epinephrine after Fasting

	Fed, Control	Period of Fast		
		12 Hours	24 Hours	48 Hours
Halothane, 2 per cent	10.9 ± 3.4* (n = 22)	5.5 ± 3.2† (n = 9)	2.2 ± 1.3† (n = 7)	2.25 ± 0.6† (n = 15)
Pentobarbital, 35 mg/kg	8.0 ± 2.2 (n = 9)	n.d.	n.d.	7.9 ± 2.97 (n = 14)

\*  $\mu\text{g}/\text{kg}$  epinephrine, mean  $\pm$  SD.† Significantly different from control values,  $P < 0.05$ .  
n.d. = not determined.

was administered intraperitoneally 10 min prior to epinephrine injection and as needed. All rats, fasted or not, received two standardized preliminary injections before the epinephrine arrhythmic threshold was determined. Each animal was given an initial dose of 0.5  $\mu\text{g}$  in order to establish catheter patency. Any increase in heart rate or blood pressure was taken to mean that the subclavian catheter was functional. Epinephrine, 6  $\mu\text{g}/\text{kg}$ , was then given to each rat. It had been determined earlier that with this dose all fasted rats showed arrhythmias while all non-fasted animals did not. The 6  $\mu\text{g}/\text{kg}$  dose served as an unbiased indicator of the direction in which to proceed in order to establish the arrhythmic threshold, *i.e.*, higher doses of epinephrine or lower doses. This practice greatly decreased the number of epinephrine injections necessary to determine the threshold. Ordinarily, once determined, the arrhythmic threshold remained stable upon repeating testing. However, in situations where numerous injections were made the arrhythmic threshold had a tendency to "drift." These animals were discarded and not used for tabulation of data.

In order to increase blood free fatty acids in fed rats, Intralipid® (Cutter Laboratories), a 10 per cent emulsion of phospholipids, palmitic, linoleic, oleic, and linolenic fatty acids, was infused intravenously at the rate of 0.1 ml/min for 30 min. After this the epinephrine threshold was tested before and after infusions as previously described. Blood ketones were increased in fed rats by infusing acetoacetic acid (10 mM/0.1 ml/min for 30 min) and the threshold tested.

Plasma free fatty acid levels were determined colorimetrically as described by Falholt *et al.*<sup>9</sup> Control and fasted rats were anesthetized with either halothane or pentobarbital and approximately 2 ml of blood removed via heart puncture. To 50  $\mu\text{l}$  of serum 1 ml of phosphate buffer and 6 ml of chloroform-heptane-methanol solution were added. After vigorous shaking for 90 sec the mixture was left standing for 15 min

and then centrifuged at 4,000 rpm for 10 min. Following this, the buffer solution was suctioned off and 5 ml of the remaining chloroform-heptane-methanol solution were shaken with 2 ml of Cu-triethanolamine for 5 min on a Vortex mixer. After centrifugation (4,000 rpm for 5 min) 3 ml of the upper phase were transfused to a test tube containing 0.5 ml of 1,5-diphenylcarbazine and mixed thoroughly. Colorimetric determination was made with a Farrand spectrophotometer at 550 nm.

Blood ketone bodies in fasted and nonfasted rats were estimated according to the method of Goschke.<sup>10</sup> Briefly, ketone acids were converted to acetone by acid hydrolysis and the amount of acetone was then determined colorimetrically. To 2 ml of heparinized blood were added 0.4 ml of barium hydroxide and 4 ml of zinc sulfate for deproteinization. Following centrifugation, a 5-ml volume of the supernatant was heated with 1 cm<sup>3</sup> of 6 M sulfuric acid in an oil bath to 110 C for 10 min. After cooling, 1 ml of potassium dichromate solution was added. The solution was then reheated to 135 C for 45 min. One ml of sodium sulfite, 1 ml of color reagent and 4 ml of carbon tetrachloride were then added and the mixture shaken for 30 min. This produced two phases, which were then separated with a separatory funnel. To the upper phase were added 6 ml of 0.25 M sodium hydroxide; the mixture was then shaken for 3 min. The concentration of acetone present was determined colorimetrically at 420 nm for amounts of more than 10  $\mu\text{g}$  and at 375 nm for amounts of less than 10  $\mu\text{g}$ .

Urinary catecholamine metabolites were determined through fluorometric analysis as described by Crout.<sup>11</sup> Forty-eight-hour urine samples were collected from rats housed in individual metabolism cages.

Statistical significance of data was determined by one-way analysis of variance, and in some cases by *t* test.

TABLE 2. Effects of Fasting on Physiologic Variables during Halothane and Pentobarbital Anesthesia

	Fed		48-hour Fasted	
	Halothane	Pentobarbital	Halothane	Pentobarbital
Mean arterial pressure (torr)	77 ± 16	138 ± 18	73 ± 10	129 ± 22
Heart rate (/min)	305 ± 62	347 ± 53	293 ± 44	313 ± 40
P <sub>O</sub> (torr)	384 ± 77	88 ± 6	400 ± 63	93 ± 4
P <sub>CO</sub> (torr)	38 ± 10	36 ± 7	36 ± 6	39 ± 6
pH	7.36 ± 0.08	7.33 ± 0.05	7.31 ± 0.06	7.37 ± 0.08

## Results

Rats anesthetized with halothane after fasting for 12, 24, and 48 hours had significantly lower epinephrine arrhythmic thresholds than animals that were not fasted (table 1). The threshold for rats anesthetized with pentobarbital was not affected by fasting.

No significant difference in the arrhythmic thresholds was detected in fed rats anesthetized with either halothane or pentobarbital. However, arrhythmic episodes during halothane anesthesia were far more severe, consisting of numerous protracted bouts of premature ventricular contractions lasting several seconds each. Arrhythmias during pentobarbital anesthesia were generally restricted to a single episode of six to ten premature ventricular contractions followed by tachycardia.

The difference between epinephrine arrhythmic thresholds in fasted and nonfasted animals was not due to variations in arterial blood pressure, heart rate, blood-gas values or pH (table 2). In addition, the threshold differences did not result from fluctuations in blood catecholamines, since analysis of urinary catecholamine metabolites from rats fasted for 48 hours showed no differences when compared with 48-hour values for normally fed rats ( $16.3 \pm 7.3$  vs.  $17.7 \pm 5.1$   $\mu\text{g}$ , mean  $\pm$  SD,  $n = 18$  each group).

Plasma free fatty acids and total ketone levels were significantly increased in fasted rats after 12-, 24-, and 48-hour fasts (table 3).

The epinephrine arrhythmic threshold was significantly decreased in rats anesthetized with halothane and given an infusion of Intralipid, but not in those given an infusion of acetoacetic acid (table 4). Fatty acid levels in the Intralipid-infused rats were found to approximate fasted values (table 3).

## Discussion

The progressive decrease of the epinephrine arrhythmic threshold in 12-, 24-, and 48-hour fasted, halothane-anesthetized rats was seen to occur concomitantly with fasting-induced increases in blood free fatty acids and ketones (table 1). There is evidence, albeit controversial, that increased levels of blood free fatty acids following myocardial infarction contribute to post-infarction arrhythmia.<sup>6,7,12</sup> In this study, infusion of Intralipid, a fatty acid emulsion, into non-fasted rats significantly lowered the epinephrine arrhythmic threshold in a way similar to fasting (table 4). This observation suggests that the lowering of the epinephrine arrhythmic threshold in fasted, halothane-anesthetized rats is in some way connected

TABLE 3. Effects of Fasting and Intralipid Infusion on Blood Free Fatty Acids and Ketones in the Rat\*

	Free Fatty Acids ( $\mu\text{mol/l}$ )	Ketones ( $\text{mg}/100 \text{ ml}$ )
Non-fasted	$0.36 \pm 0.24$ ( $n = 14$ )	$9.8 \pm 3$ ( $n = 9$ )
Fasted 12 hours	$1.07 \pm 0.48^\dagger$ ( $n = 13$ )	$17.9 \pm 5^\dagger$ ( $n = 7$ )
Fasted 24 hours	$0.61 \pm 0.23^\dagger$ ( $n = 13$ )	$18 \pm 2^\dagger$ ( $n = 10$ )
Fasted 48 hours	$0.68 \pm 0.31^\dagger$ ( $n = 13$ )	$23.0 \pm 2^\dagger$ ( $n = 8$ )
Non-fasted, Intralipid infused	$0.99 \pm 0.15^\dagger$ ( $n = 8$ )	—

\* Values represent means  $\pm$  SD.

$^\dagger$  Significantly different from non-fasted value,  $P < 0.01$ .

TABLE 4. Effects of Intralipid, Acetoacetic Acid, and Saline Infusion on the Epinephrine Arrhythmic Threshold of Halothane-anesthetized, Non-fasted Rats

Saline solution (0.1 ml/min for 30 min)	$10.2 \pm 2.8^*$	( $n = 6$ )
Intralipid, 10 per cent (0.1 ml/min for 30 min)	$2.7 \pm 1.1^\dagger$	( $n = 6$ )
Acetoacetic acid (10 mM/0.1 ml/min for 30 min)	$10.4 \pm 2.1$	( $n = 6$ )

\*  $\mu\text{g}/\text{kg}$  epinephrine, mean  $\pm$  SD.

$^\dagger$  Significantly different from saline-infused values,  $P < 0.01$ .

to increased blood free fatty acids or lipid metabolism. Possibly, fasting-induced changes in myocardial metabolism may render the heart more susceptible to arrhythmia-inducing drugs. Fasting in the rat has been shown to cause a shift in myocardial metabolism from utilization of mixed carbohydrate-lipid-substrate to utilization of predominantly lipid substrate.<sup>13</sup> It is thought that this shift is brought about through inhibition of glycolysis by the increased levels of blood free fatty acids. Without a viable glycolytic pathway, halothane may further disrupt myocardial metabolism in the fasted heart by inhibiting other aspects of energy metabolism. Some believe that halothane acts as a detergent on cell and mitochondrial membranes, causing them to become more fluid and expand.<sup>14</sup> Expansion of cellular membranes could cause disruption of membrane action potentials, resulting in arrhythmia. It should be pointed out, however, that there is no direct evidence to support the hypothesis that halothane produces its adverse myocardial effects through direct membrane interaction.

Another possible explanation for the enhanced arrhythmogenicity in the fasted, halothane-anesthetized rat may be based on the fact that the fasted heart is obliged to extract more blood free fatty acids for metabolic purposes. Studies have shown that myocardial metabolism of free fatty acids exclusive of other substrates increases oxygen requirements of the heart, but does not increase mechanical activity.<sup>15</sup>

Mjøs *et al.* demonstrated that the severity of acute myocardial ischemic injury in dogs is positively correlated with myocardial extraction of free fatty acids.<sup>16</sup> In our study, exclusive metabolism of free fatty acids by the fasted heart during halothane anesthesia may have increased oxygen demand without a corresponding increase in oxygen delivery. A functional oxygen deficit could result in arrhythmia. However, there is evidence that the halothane-anesthetized heart is not ischemic.<sup>17</sup> Also arguing against an oxygen-deficit hypothesis is the observation that the maximum increases in blood free fatty acids occurred after 12 hours of fasting, while the lowest epinephrine arrhythmic threshold did not develop until after 48 hours of fasting (table 1). It would seem that mere increase in blood free fatty acids is not the only factor operating to increase myocardial irritability through fasting. Perhaps there are temporal changes in myocardial lipid metabolism that do not become fully manifest until after 12 hours of fasting.

The effect of fasting on the epinephrine arrhythmic threshold of halothane-anesthetized rats may not have been the direct result of blood substrate or myocardial metabolic changes, but may have been due to some nonspecific stress factor such as heightened sympathetic nervous activity. However, analysis of blood pressure, heart rates, blood pH, and urinary catecholamine excretions revealed no significant difference between fasted and non-fasted animals (table 2). Finally, if a nonspecific stress factor caused the lowering of the epinephrine arrhythmic threshold in halothane-anesthetized rats, why did it not affect the arrhythmic threshold in pentobarbital-anesthetized animals? While halothane-epinephrine arrhythmia and pentobarbital-epinephrine arrhythmia are not necessarily of the same etiology, it would not be unreasonable to expect that a nonspecific stress factor should have also lowered the pentobarbital arrhythmic threshold. Since this was not seen, it would appear that the arrhythmia-potentiating effects of fasting are dependent in some way on the presence of halothane.

Surprisingly, there was no significant difference among the epinephrine arrhythmic thresholds for non-fasted and halothane- and pentobarbital-anesthetized rats. This observation seems to be in apparent contradiction to the prevailing notion that halothane "sensitizes" the heart to epinephrine more than do other anesthetics. However, the criterion for the epinephrine arrhythmic threshold in this study was simply the occurrence of four or more premature ventricular contractions following epinephrine injection. No allowance was made for the duration of ar-

rhythmic episodes. It was evident throughout the course of this study that the arrhythmias during pentobarbital anesthesia were generally limited to single short clusters of premature ventricular contractions, followed by tachycardia, while arrhythmias during halothane anesthesia consisted of multiple bouts lasting several seconds each. Although no effort was made to include a measure of severity as an indicator of epinephrine-induced irritability, it was evident that halothane produced a more serious arrhythmic situation. In addition, the significantly higher blood pressure in pentobarbital-anesthetized animals prior to epinephrine injection may have helped to lower the epinephrine arrhythmic threshold (table 2). There is evidence demonstrating that increased blood pressure predisposes to production of arrhythmias during anesthesia.<sup>2</sup> It has been suggested that increased blood pressures cause stretching of myocardial Purkinje fibers, resulting in slowing of conduction velocity and an increase in the rate of diastolic depolarization.<sup>3,18</sup> Both of these effects could predispose the heart to arrhythmias.

In conclusion, the data from this study demonstrate that fasting or infusion of lipids into non-fasted rats lowers the epinephrine arrhythmic threshold during halothane anesthesia. Although circumstantial, the evidence suggests that some aspect of myocardial lipid metabolism may be involved. In view of the common practice of fasting patients before operation, further investigation into the relationship of nutrition to spontaneous cardiac arrhythmias during halothane anesthesia seems warranted.

The authors thank Ms. A. Jozefiak and Mr. D. Visintine for technical assistance in this study.

## References

1. Katz RL, Bigger JF: Cardiac arrhythmias during anesthesia and operation. *ANESTHESIOLOGY* 33:193-213, 1970
2. Dresel PE, Sutter MC: Factors modifying cyclopropane-epinephrine cardiac arrhythmias. *Circ Res* 9:1284-1290, 1961
3. Zink J, Sasyniuk BI, Dresel PE: Halothane-epinephrine-induced cardiac arrhythmia and the role of heart rate. *ANESTHESIOLOGY* 43:548-555, 1975
4. Davis LD, Helmer PR, Murphy PR: Role of potassium in cyclopropane-epinephrine ventricular tachycardia. *ANESTHESIOLOGY* 25:54-58, 1964
5. Hashimoto K, Hashimoto K: The mechanism of sensitization of the ventricle to epinephrine by halothane. *Am Heart J* 83:652-658, 1972
6. Rowe MJ, Neilson JMM, Oliver MF: Control of ventricular arrhythmias during myocardial infarction by antilipolytic treatment using nicotinic acid. *Lancet* 1:295-300, 1975

7. Gupta DK, Young T, Jewitt DE, et al: Increased free fatty acid concentrations and their significance in patients with acute myocardial infarction. *Lancet* 2:1209-1213, 1969
8. Zahed B, Miletich DJ, Ivankovich AD, et al: Arrhythmic doses of epinephrine, and dopamine during halothane, enflurane, methoxyflurane and fluroxene anesthesia in goats. *Anesth Analg (Cleve)* 56:207-210, 1977
9. Falholt K, Lund B, Falholt W: An easy colorimetric micro-method for routine determination of free fatty acids in plasma. *Clin Chim Acta* 46:105-111, 1973
10. Goschke H: Estimation of ketone bodies in blood, cerebrospinal fluid and urine. *Clin Chim Acta* 28:359-364, 1970
11. Crout JR: Catecholamines in urine, *Standard Method of Clinical Chemistry*; Edited by Seligson D. New York and London, Academic Press, 1961, pp 62-80
12. Kostis JB, Mavrogeorgis EA, Horstmann E, et al: Effect of high concentrations of free fatty acids on the ventricular fibrillation threshold of normal dogs and dogs with acute myocardial infarction. *Cardiology* 58:89-98, 1973
13. Randle PJ, Newsholme EA, Garland PB, et al: Regulation of glucose uptake by muscle. 8. Effects of fatty acids, ketone bodies and pyruvate and of alloxan-diabetes and starvation, on the uptake and metabolic fate of glucose in rat heart and diaphragm muscle. *Biochem J* 93:652-665, 1964
14. Makelainen A, Nikki P, Vapaatalo H: Halothane induced lipolysis in rats. *Acta Anesthesiol Scand* 17:170-177, 1973
15. Challoner DR, Steinberg D: Effects of free fatty acids on the oxygen consumption of perfused rat heart. *Am J Physiol* 210: 280-286, 1966
16. Mjøs OD, Miller MB, Riemersma RA, et al: Effects of p-chlorophenoxyisobutyrate on myocardial free fatty acid extraction, ventricular blood flow, and epicardial ST-segment elevation during coronary occlusion in dogs. *Circulation* 53:494-500, 1976
17. Merin RG, Kumazawa F, Lika NL: Myocardial function and metabolism in the conscious dog during halothane anesthesia. *ANESTHESIOLOGY* 44:402-415, 1976
18. Vick RL: Effect of altered heart rate on chloroform-epinephrine cardiac arrhythmias. *Circ Res* 18:316-322, 1966