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Influence of Anesthetic Agent on Survival Following Hemorrhage

David E. Longnecker, M.D., and Benjamin C. Sturgill, M.D.†*

One hundred and twenty-eight Sprague-Dawley rats were each anesthetized with one of four anesthetics and subjected to a standard hemorrhage protocol to determine the effects of anesthetics on survival following hemorrhage. The anesthetics studied were: halothane, 1.26 vol per cent; fluroxene, 4.5 vol per cent; pentobarbital, 50 mg/kg, ip; ketamine, 125 mg/kg, im. Mean arterial pressure was controlled at 40 torr during 60 minutes of hemorrhage. Cumulative survival rates were determined at the end of hemorrhage and 24 hr, 48 hr, 72 hr, and 7 days after hemorrhage. Twenty-four-hour survival rates were: halothane, 50.0 per cent; fluroxene, 56.3 per cent; pentobarbital, 59.4 per cent; ketamine, 84.4 per cent. Seven-day survival rates were: halothane, 46.9 per cent; fluroxene, 18.7 per cent; pentobarbital, 53.1 per cent; ketamine, 81.3 per cent. Survival rates were significantly higher ($P < 0.05$) in animals anesthetized with ketamine compared with survival rates associated with the other anesthetics. Long-term (72-hr and seven-day) survival rates were significantly ($P < 0.05$) lower in animals anesthetized with fluroxene. Microscopic examination of livers and small intestines revealed significantly fewer ($P < 0.05$) pathologic changes in the splanchnic organs of hemorrhaged rats anesthetized with ketamine compared with animals anesthetized with the other drugs. (Key words: Hemorrhage, anesthetic and survival; Anesthetics, volatile, halothane; Anesthetics, volatile, fluroxene; Anesthetics, intravenous, ketamine; Hypnotics, barbiturate, pentobarbital.)

* Associate Professor of Anesthesiology.

† Professor of Pathology.

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Address reprint requests to Dr. Longnecker: Department of Anesthesiology, University of Virginia Medical Center, Box 238, Charlottesville, Virginia 22903.

SEVERAL INDEPENDENT OBSERVATIONS suggest that anesthetics might influence survival following hemorrhagic hypotension. Chien reported that an intact sympathetic nervous system increased the survival rates of unanesthetized dogs subjected to hemorrhage.[1] However, augmented sympathoadrenal activity appears to be detrimental during prolonged hemorrhagic hypotension, since both survival times[2] and survival rates[3] were reduced in dogs treated with intravenous infusion of norepinephrine during hemorrhage. Since anesthetics alter sympathetic activity,[4–7] it is possible that survival rates of animals subjected to hemorrhage may be altered by anesthetics by their action on the sympathetic nervous system.

The present study was undertaken to determine the influences of two inhalation anesthetics, barbiturate anesthesia, and ketamine anesthesia on survival in rats subjected to hemorrhage. Histologic examination of several organs was performed to evaluate the effects of the anesthetics on organ morphology following hemorrhage.

Methods

One hundred and twenty-eight male Sprague-Dawley rats (weights 117 ± 3 g; mean ± SE) were divided into four groups of 32 animals each. Anesthesia was established with one of the following agents: halothane, 1.26 vol per cent; fluroxene, 4.55 vol per cent; pentobarbital, 50 mg/kg, ip; ketamine, 125 mg/kg, im. All animals breathed room air during anesthesia. Inhaled concentrations of the volatile agents were determined at 15-minute intervals by gas chromatography.‡ The

‡ Gow-Mac Model 750 Flame Ionization Detector with 6-foot stainless steel column containing 20 per cent SE-30 on 80–100 mesh Chromasorb W.

TABLE 1. Average (Mean ± SE) Body Weights, Mean Arterial Pressures (MAP), and Heart Rates (HR) during Anesthesia Prior to Hemorrhage

	Pentobarbital (n = 32)	Halothane (n = 32)	Fluroxene (n = 32)	Ketamine (n = 32)	Significance (Analysis of Variance)
Body weight (g)	114 ± 4	113 ± 3	121 ± 2	119 ± 2	NS
MAP (torr)	96 ± 3	71 ± 1	79 ± 1	97 ± 2	$P < 0.005$
HR (beats/min)	475 ± 6	402 ± 8	381 ± 6	463 ± 6	$P < 0.005$
Maximum shed blood volume during hemorrhage (ml/100 g)	2.3 ± 0.1	2.6 ± 0.1	3.1 ± 0.1	3.4 ± 0.1	$P < 0.005$

inhaled concentrations represent approximate MAC values for these agents in young rats.[8,9] The injectable agents were supplemented with a third of the initial anesthetic dose as indicated to prevent purposeful movements of the rats during the experimental protocol. The animals were placed on a heating pad to maintain rectal temperatures at 36–37 C.

A polyethylene catheter (PE 50) was placed in the left carotid artery and connected to a pressure transducer (Statham P23Dc) and a servo-controlled syringe system for measuring arterial pressure and inducing hemorrhage. A volume output from the servo-controlled syringe provided a continuous monitor of the shed blood volume during hemorrhage. Standard limb leads of the ECG were utilized to obtain heart rate. Mean arterial pressure, heart rate, and shed blood volume were measured continuously and recorded at 5-minute intervals.

The protocol consisted of a 30-minute period of stable anesthesia, a 60-minute period of hypotension during which mean arterial pressure was controlled at 40 torr, a 5-minute period for reinfusion of shed blood, and an additional 30-minute post-hemorrhage control period. Then the carotid artery was ligated, the neck wound closed with stainless steel clips, and anesthetic administration was discontinued. The rats were placed in individual heated cages until recovery from anesthesia was complete, when they were transferred to individual cages and allowed food and water *ad libitum*. They were observed for survival at regular intervals over the next seven days. Survival rates were determined at the termination of the hemorrhage period (60 minutes) and thereafter at 24-hour intervals. Cumulative survival rates were obtained at each time interval by expressing the total number of survivors as percentage of the number of animals originally in each group.

Twenty-five animals received three MAC-hours (4.5 per cent fluroxene for 3 hours) of fluroxene without hemorrhage to determine the effect of fluroxene alone on survival.

Four animals that died between 24 hours and seven days after hemorrhage were selected from each group for microscopic histologic examination. Organs examined included the heart, lungs, liver, spleen, kidneys, and small intestine (jejunum) of each animal. The organs were removed from the animal, sectioned, and immediately placed in formalin. Microscopic sections were stained with hematoxylin and eosin. Histologic sections were identified by number only and examined at a single time by an experienced pathologist (BCS) who was unaware of the anesthetic relationship of the specimen. Pathologic changes were graded according to the following scale: 0 = no pathologic change; 1 = mild changes; 2 = moderate changes; 3 = severe or extensive changes.

Frequency data (survival rates) were analyzed by the chi-square test. Quantitative data (heart rate, blood pressure, graded pathologic changes in organs) were analyzed by analysis of variance. When differences were obtained by analysis of variance, Duncan's multiple range test[10] (the Q statistic) was utilized to determine individual differences between groups.

Results

Table 1 summarizes the values for body weight, mean arterial pressure, and heart rate for the four groups during the initial anesthetic period. Prior to hemorrhage, mean arterial

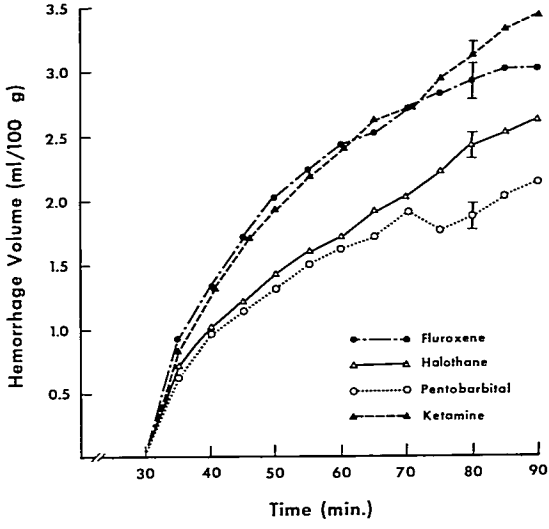

FIG. 1. Average shed blood volume (± standard error) during hemorrhage for each of the four groups. Shed blood volumes were greater ($P < 0.05$) with fluroxene or ketamine than with halothane or pentobarbital anesthesia.

pressure was lowest during halothane anesthesia ($P < 0.05$ compared with the other anesthetics). Arterial pressure was significantly lower during fluroxene anesthesia compared with ketamine or pentobarbital anesthesia ($P < 0.05$). Heart rates were significantly slower in animals anesthetized with halothane or fluroxene compared with those anesthetized with pentobarbital or ketamine ($P < 0.05$).

During hemorrhage the maximum shed blood volume was significantly larger ($P < 0.05$) in animals anesthetized with ketamine or fluroxene compared with those anesthetized with halothane or pentobarbital (table 1). The time courses for hemorrhage volume are illustrated in figure 1. The heart rates during hemorrhage were not significantly different among groups.

Table 2 depicts survival rates for the four groups. Statistically significant ($P < 0.05$) differences in survival rates were observed from the end of hemorrhage (60 minutes) throughout the seven-day observation period. The survival rate was highest in animals anesthetized with ketamine (81.3 per cent), while the fewest survivors were associated with fluroxene anesthesia (18.7 per cent). Pentobarbital and halothane resulted in intermediate survival rates following hemorrhage. Survival rates following hemorrhage in animals receiving ketamine were significantly ($P < 0.05$) higher at each time compared with survival rates of animals receiving fluroxene, pentobarbital, and halothane anesthesia. Survival rates for fluroxene versus pentobarbital or halothane were not different initially after hemorrhage, but significant differences ($P < 0.05$) were observed from 72 hours through seven days after hemorrhage. The survival rate was 100 per cent in animals exposed to 4.5 per cent fluroxene for 3 hours (3 MAC hours) without hemorrhage.

Table 3 summarizes the severities of the pathologic lesions among the four groups. The most striking pathologic lesions occurred in the small intestine (jejunum) and in the liver. In the gut, loss of epithelium and sloughing and necrosis of the villi were prominent after hemorrhage in animals anesthetized with pentobarbital, halothane, and fluroxene, but were significantly ($P < 0.5$) less evident in animals receiving ketamine

TABLE 2. Cumulative Survival Rates at Various Times after Onset of Hemorrhage

Time	Survival Rate (Per Cent)				Significance (Chi-square)
	Pentobarbital (n = 32)	Halothane (n = 32)	Fluroxene (n = 32)	Ketamine (n = 32)	
60 min	71.9	59.4	78.1	96.9	$P < 0.01$
24 hr	59.4	50.0	56.3	84.4	$P < 0.05$
48 hr	56.3	46.9	37.5	84.4	$P < 0.01$
72 hr	56.3	46.9	25.0	81.3	$P < 0.001$
7 days	53.1	46.9	18.7	81.3	$P < 0.001$

anesthesia. Comparison of individual groups revealed significant differences ($P < 0.05$) between the ketamine group and all of the other groups.

In the liver, microscopic changes consisted primarily of erythrocyte engorgement and congestion. However, three of four rats anesthetized with fluroxene had moderate to extensive midzonal hepatic necrosis. Significant differences ($P < 0.05$) in severities of hepatic changes were observed among groups. Individual comparisons revealed that significantly fewer severe ($P < 0.05$) hepatic changes were associated with hemorrhage in animals receiving ketamine than in animals anesthetized with either pentobarbital or fluroxene. Fluroxene was associated with significantly more ($P < 0.05$) hepatic changes than was halothane. Fluroxene and pentobarbital were not significantly different from each other with regard to hepatic lesions.

Congestion and infrequent focal tubular degeneration were observed in the kidneys. Pulmonary lesions consisted of mild congestion, perivascular edema, and atelectasis. No significant histologic difference in either organ was found among the groups. Similarly, there was no significant difference in myocardial or splenic abnormalities among the groups.

Discussion

The most prominent results of this study were the higher survival rates following hemorrhage in rats anesthetized with ketamine compared with the other anesthetics, and the relative absence of splanchnic (small intestine and liver) pathologic changes after hemorrhage in animals receiving ketamine. The results cannot be explained by differences in shed blood volumes, since the highest survival rate occurred in animals subjected to the largest blood-volume reductions during hemorrhage.

The anesthetics tested in the present study were selected for specific reasons. Since sympathetic nervous system activity is reported to alter survival following hemorrhage,[1-3] halothane and fluroxene were studied as examples of anesthetics associated with decreased and increased sympathetic nervous system activity, respectively.[4,5] Ketamine was studied because of its purported benefits for patients in hemorrhagic shock, despite any

TABLE 3. Pathologic Changes (Mean ± SE) in Organs of Four Rats in Each Group*

	Pentobarbital	Halothane	Fluroxene	Ketamine	Significance (Analysis of Variance)
Small intestine	2.25 ± 0.25	2.25 ± 0.25	2.50 ± 0.29	0.50 ± 0.50	$P < 0.005$
Liver	2.00 ± 0.41	1.25 ± 0.25	2.75 ± 0.25	1.00 ± 0.41	$P < 0.005$
Lung	1.75 ± 0.25	1.75 ± 0.25	2.00 ± 0.41	1.75 ± 0.25	NS
Kidney	1.50 ± 0.50	0.50 ± 0.50	0	0	NS
Spleen	0.50 ± 0.50	0	1.00 ± 0.58	0	NS
Heart	1.00 ± 0.58	0.50 ± 0.50	0	0.50 ± 0.50	NS

* Pathologic changes were scored as follows: 0 = no pathologic change; 1 = mild changes; 2 = moderate changes; 3 = severe or extensive changes.

firm laboratory or clinical data to support this recommendation.[11,12] Pentobarbital was studied because of its frequent use in the laboratory during hemorrhagic shock investigations. In addition, Kovach *et al.*[13] demonstrated increased survival times in dogs subjected to hemorrhage during maintenance of cerebral blood flow from a donor dog by a cross-perfusion technique. Silver documented cerebral tissue hypoxia in rats during controlled hemorrhage to a mean arterial pressure of 30 torr for 60 minutes.[14] Since barbiturates appear to protect the brain during focal ischemia,[15] it seemed possible that barbiturates might also improve survival associated with hemorrhage. However, our results provided no evidence to support this hypothesis.

Survival results following hemorrhage during fluroxene anesthesia may have been affected by metabolism of the drug, since fluroxene undergoes substantial biotransformation to potentially toxic metabolites in rats.[16] Our observations of midzonal hepatic necrosis following fluroxene plus hemorrhage were similar to the changes observed by Harrison and Smith[16] in enzyme-induced rats exposed to fluroxene without hypovolemia. They observed no mortality from fluroxene in rats not pretreated with enzyme-inducing agents. The absence of mortality in the 25 rats exposed to fluroxene without hemorrhage in the present study suggests that a mechanism other than toxicity from the biotransformation of fluroxene may have accounted for the increased mortality in hemorrhaged animals anesthetized with fluroxene. However, the possible role of biotransformation in influencing survival following fluroxene and hemorrhage is not eliminated by the present study.

The higher survival rate in rats subjected to hemorrhage while anesthetized with ketamine was dramatic (31 of 32 animals survived the hemorrhage period). The increased survival correlates well with the lack of microscopic changes in the organs of hemorrhaged animals anesthetized with ketamine. Numerous previous studies have suggested that the splanchnic viscera represent a "target organ" during hemorrhage, and that the underlying mechanism of splanchnic pathologic changes is cellular hypoxia. Lillehei[17] used a cross-perfusion technique to maintain intestinal blood flow during hemorrhage in dogs and reduced the mortality rate associated with hemorrhage from 90 per cent to 10 per cent. Haglund[18] reported that intraluminal perfusion of the cat small intestine with oxygenated saline solution during hemorrhage prevented the development of intestinal mucosal lesions similar to those described in the present study. The present observations suggest that splanchnic tissue hypoxia is less evident with hemorrhage during ketamine anesthesia, and this may account for the better survival in these animals.

Theye and co-workers[19] compared survival times in three groups of dogs subjected to graded hemorrhage. Animals were anesthetized with equipotent concentrations of cyclopropane, isoflurane, or halothane. Survival times were longest with halothane (247 minutes) and shortest with cyclopropane (146 minutes). The results were consistent with the hypothesis that anesthetics that allow longer survival during hemorrhage are those associated with decreased sympathetic activity. Despite the theoretical objection to ketamine on the basis that it may increase sympathetic activity, the present results suggest that the key factor in determining survival following hemorrhage may be the regional distribution of blood flow and the balance between oxygen delivery and oxygen demand, not sympathetic nervous activity *per se*. Ketamine appears to minimize the development of splanchnic ischemia during hemorrhage, either by maintaining oxygen delivery or by decreasing oxygen demand, and this mechanism probably accounts for the higher survival rates seen with this agent in the present study.

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Pulmonary Physiology

HYPOXIC VASOCONSTRICTION In awake man, alveolar hypoxia results in regional pulmonary vasoconstriction. This tends to minimize the effects of local underventilation on systemic arterial oxygenation. The authors have shown that pulmonary vascular reactivity is decreased when cats are anesthetized with clinical concentrations of halothane, trichloroethylene, and diethyl ether. The present study examined the response to hypoxia of the isolated cat lung preparation in the presence of various concentrations of methoxyflurane (MOF). MOF alone produced a dose-dependent decrease in pulmonary vascular resistance. The increase in pulmonary vascular resistance normally observed in the presence of hypoxia (produced by administration of 3 per cent carbon dioxide and 3 per cent oxygen in nitrogen for three minutes) was attenuated following administration of MOF. Pulmonary vascular reactivity returned to control values when MOF was discontinued. The response was dose-dependent (0.2 per cent MOF produced a 39 per cent decrease in response; 0.5 per cent MOF produced an 80 per cent decrease in response; when concentrations greater than 1 per cent were administered no response was present). These data tend to confirm the observation that general anesthesia decreases the response to inhalational hypoxia. *(Sykes MK, and others. The effect of methoxyflurane on pulmonary vascular resistance and hypoxic pulmonary vasoconstriction in the isolated perfused cat lung. Br J Anaesth 48: 191–194, 1976.)*