

Isoflurane and Enflurane-induced Hepatic Necrosis in Triiodothyronine-pretreated Rats

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Exposure of triiodothyronine (T_3)-pretreated rats to 1.3% isoflurane, 1.8% enflurane, or 1% halothane in 21% oxygen (air) for two hours resulted in hepatic centrilobular necrosis. The incidence of the liver lesion was 28, 24, and 92% after exposure to isoflurane, enflurane, and halothane, respectively. Histopathologic grading indicated that the necrosis was more severe after halothane than after isoflurane or enflurane anesthesia. No lesion was observed in livers prepared from non-anesthetized T_3 -pretreated rats or in livers prepared from rats which were pretreated with the vehicle for T_3 and then anesthetized with either isoflurane, enflurane, or halothane. Hepatic necrosis was not observed in vehicle-treated rats exposed to isoflurane in 12% oxygen or in vehicle-treated rats that were deprived of food for 12 hours prior to exposure to isoflurane under hypoxic conditions. Food restriction to maintain the body weight gain of vehicle-treated rats similar to that of T_3 -treated rats did not result in hepatotoxicity after exposure to halothane in 21% oxygen. Liver necrosis did not occur in pentobarbital anesthetized (40 mg/kg, intraperitoneally) T_3 -pretreated rats. These results indicate that isoflurane and enflurane, like halothane, can induce hepatic centrilobular necrosis in T_3 -pretreated rats. The mechanism for liver toxicity of these volatile anesthetic agents in this model remains to be determined. (Key words: Anesthetics, volatile: isoflurane, enflurane, halothane. Hormones: thyroid. Hypnotics: barbiturates, pentobarbital. Liver: hepatotoxicity. Toxicity: hepatic.)

THE HYPERTHYROID STATE has been shown to potentiate hepatotoxicity in rodents caused by chloroform,¹ carbon tetrachloride,² 1,1-dichloroethylene,³ and acetaminophen.⁴ Wood *et al.*⁵ have reported that hepatic centrilobular necrosis develops in rats pretreated with

triiodothyronine (T_3), and then anesthetized with 1% halothane for two hours in air, but did not observe a lesion in the livers from six hyperthyroid rats exposed to enflurane. Using a different dose and route of administration of T_3 and a longer pretreatment schedule to render rats hyperthyroid, we repeated these experiments, using isoflurane instead of enflurane, with the expectation that isoflurane, like enflurane would not induce liver necrosis. We now report that both isoflurane and enflurane induce hepatic necrosis in hyperthyroid rats, but at a much lower frequency and severity than halothane.

Materials and Methods

Male Sprague-Dawley rats,[¶] weighing 100 to 125 g were given T_3 , 5 mg/kg or 2.5 ml/kg of the vehicle for T_3 (3.0 mM sodium hydroxide in physiologic saline solution) intraperitoneally, daily for six days. The rats were weighed daily throughout the pretreatment period, and serum T_3 concentrations were measured⁶ in six rats given T_3 24 hours after their last pretreatment dose. On day seven, rats in groups of five or six were selected randomly for exposure to either isoflurane or halothane (experiment 1), enflurane or halothane (experiment 2), or given pentobarbital or exposed to halothane (experiment 3). The regimen used for pretreating and anesthetizing the animals is shown in table 1. Rats pretreated with T_3 and exposed to either isoflurane, enflurane, or given pentobarbital were matched with a similarly pretreated number of T_3 -pretreated rats exposed to halothane. On separate days, litter mates which also had been pretreated with T_3 were exposed to air in the anesthetic chamber (experiment 4).

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Received from the Department of Anesthesiology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232. Accepted for publication June 10, 1982.

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In an attempt to control for the metabolic stress induced by the hyperthyroid state, we performed an experiment to determine if other metabolic stresses would sensitize the liver to anesthetic-induced necrosis. We pretreated 40 rats with the vehicle for T_3 as described previously and exposed them, in groups of five or six to 1.3% isoflurane in 12% oxygen for two hours and 86.7% nitrogen with half the animals deprived of food 12 hours prior to the anesthetic exposure. Six hours after anesthesia, the livers were removed for histologic examination.

The reduced ratio of body weight gain to food eaten has been used as a sign of hyperthyroidism in the rat.⁵ To distinguish between the effects of food intake and weight gain from the effects of T_3 in sensitizing the liver to anesthesia-induced hepatic necrosis, the gain in body weight of 10 vehicle-treated rats was maintained similar to that of 10 T_3 -treated rats during the pretreatment period. This was accomplished by restricting the quantity of food available to the vehicle-pretreated rats to an amount (in grams) equal to the gain in body weight (in grams) of T_3 -pretreated rats during the previous 24 hours. The rats were then exposed in groups of five, to 1% halothane in 21% oxygen (USP compressed air) for two hours and the liver removed for histologic examination six hours after anesthesia.

Sixty liter glass sided aquaria with an inlet and outlet port at opposite ends were used as the anesthetic chambers. No more than 12 rats per chamber were either anesthetized with volatile agents or exposed to USP

TABLE 2. Net Increase in Body Weight (mean \pm SEM) of Rats Pretreated with Triiodothyronine (T_3)

Pretreatment	Net Increase (g)	Per Cent Increase Compared with the Vehicle-treated Rats at the End of Pretreatment
T_3 (n = 295)	8.3 \pm 0.6*	16
Vehicle (n = 72)	46.0 \pm 0.7	

* $P < 0.001$ compared with vehicle (Student's t test).
n = number of rats.

compressed air in the absence of anesthetic vapors. Air flow into the chambers was at the rate of 10 l/min. A Fluotec-3** vaporizer which had been cleaned thoroughly and found free of halothane as determined by gas chromatography was calibrated for the vaporization of isoflurane. Halothane was vaporized using a Fluotec-3 vaporizer, and vaporization of enflurane was accomplished with an Ethrane plenum†† vaporizer. The oxygen concentration within the chambers was monitored continuously using a polarographic oxygen analyzer,‡‡ and at 15- to 30-min intervals samples from within the chambers were analyzed for the concentration of the volatile agents by gas chromatography.

Six hours after the inhalational exposure, or six hours after recovery from pentobarbital (spontaneous return of the righting reflex), the rats were killed by decapitation. The livers were removed and liver slices 1 to 2 mm thick were fixed in 10% buffered formalin pH 7.0, dehydrated in graded strength ethanol, cleared in xylol, and embedded in paraffin. Sections 8 μ m thick were stained with hematoxylin and eosin, and necrosis was evaluated by light microscopy. Histopathologic grading was performed by a pathologist who was unaware of the treatment received by the rats.

The data were analyzed using Student's t test and χ^2 analysis with Yates correction.

Results

The T_3 concentration in the serum of six T_3 -treated rats on the seventh day immediately before induction of anesthesia after six daily injections of T_3 5 mg/kg was $20,240 \pm 2,590$ ng/dl (mean \pm SEM). During the pretreatment period, rats given T_3 gained less weight as a result of their hyperthyroid state than rats given the vehicle for T_3 (table 2). The mortality of T_3 -pretreated rats exposed to halothane was significantly greater than that resulting from exposure to isoflurane and enflurane, or from the administration of pentobarbital (table 3). Enflurane caused a significantly less mor-

TABLE 1. Anesthetic Regimen

Pretreatment	Total Number of Rats	Anesthetic
Experiment 1		
T_3	45	1.3% Isoflurane, 21% O ₂ *, 2 hours
Vehicle	18	Same
T_3	45	1% Halothane, 21% O ₂ , 2 hours
Vehicle	18	Same
Experiment 2		
T_3	40	1.8% Enflurane, 21% O ₂ , 2 hours
Vehicle	18	Same
T_3	45	1% Halothane, 21% O ₂ , 2 hours
Vehicle	18	Same
Experiment 3		
T_3	42	Pentobarbital, 40 mg/kg, ip, 21% O ₂ , 2 hours
T_3	42	1% Halothane, 21% O ₂ , 2 hours
Experiment 4		
T_3	36	21% O ₂ , 2 hours

* USP Compressed Air.

** Cyprane, North America Inc., Tonawanda, New York.

†† Fraser-Sweatman Inc., Lancaster, New York.

‡‡ Ohio 200 Oxygen Monitor, Ohio Medical Products, Madison, Wisconsin.

tality rate in T₃-pretreated rats than did pentobarbital, but the mortality rate between isoflurane and pentobarbital was not significantly different. Deaths occurred during the two-hour period of anesthesia with the volatile agents and within three hours after the administration of pentobarbital. No deaths occurred in the non-anesthetized T₃-pretreated rats or in anesthetized rats that received the vehicle for T₃.

Centrilobular necrosis occurred in the livers of T₃-pretreated rats anesthetized with either isoflurane, enflurane, or halothane. Necrosis was not observed in the periportal regions. As shown in table 4, the incidence and severity of the lesion was markedly less after isoflurane or enflurane than after halothane anesthesia. No liver necrosis occurred in pentobarbital-anesthetized T₃-pretreated rats despite the observation that the duration of anesthesia in these animals varied from 4.75 to 5.5 h, the interval from spontaneous loss to spontaneous return of the righting reflex. Recovery from two hours of anesthesia with the volatile agents occurred within 20 min. No hepatic necrosis was observed after anesthesia in rats pretreated with the vehicle for T₃ (total number of rats = 72) or in non-anesthetized T₃-pretreated rats.

No hepatic lesion was observed in the livers from 20 vehicle-pretreated rats anesthetized with isoflurane in 12% oxygen, nor did a liver lesion develop in the livers from 20 vehicle-pretreated rats that were deprived of food 12 hours prior to being anesthetized with isoflurane in 12% oxygen.

No liver lesion was found in ten halothane-anesthetized vehicle-treated rats when food intake was restricted so that their body weight gain from 107 ± 2.3 to 114.6 ± 2.8 g (mean \pm SEM) was similar to that of ten T₃-treated rats, from 108.2 ± 1.9 to 117.5 ± 2.0 g during the pretreatment period. Hepatic necrosis, however, was observed in the T₃-treated rats anesthetized with halothane.

Discussion

The concentration of T₃ found in the serum of rats immediately before induction of anesthesia reflects the

TABLE 3. Per Cent Mortality of Triiodothyronine-treated Rats during Isoflurane, Enflurane, Halothane, and Pentobarbital Anesthesia

	Anesthetic Agent				
	Isoflurane	Enflurane	Halothane	Pentobarbital	Air
Per cent mortality	11.1	5.0	32.6	15.3	0
Number of rats	45	40	132	42	36

P value (χ^2 analysis): isoflurane vs. halothane < 0.001 , isoflurane vs. enflurane NS, isoflurane vs. pentobarbital NS, enflurane vs. halothane < 0.001 , enflurane vs. pentobarbital < 0.05 , halothane vs. pentobarbital < 0.01 .

TABLE 4. Incidence and Severity of Hepatic Centrilobular Necrosis in Triiodothyronine Pretreated Rats after Anesthesia

Histopathologic Grade*	Anesthesia				
	Isoflurane (40)†	Enflurane (38)	Halothane (89)	Pentobarbital (36)	Air (36)
0	29	29	7	36	36
1/2+	4	4	2		
1+	5	4	28		
2+	2	1	46		
3+	0	0	6		
4+	0	0	0		
Total incidence %	28‡	24‡	92	0	0

* 0 = no lesion; 1/2+ = necrotic cells at the first cell level away from the central vein and hyaline degeneration present; 1+ = necrotic cells extruding two to three cell levels away from the central vein; 2+ = necrotic cells extruding three to six cells away from the central vein; 3+ = necrosis extending from one central vein to another; 4+ = extensive centrilobular necrosis throughout the section.

† Numbers in parentheses indicate the total number of rats.

‡ $P < 0.001$ isoflurane compared to halothane and enflurane compared with halothane (χ^2 analysis).

six days of pretreatment with T₃ (5 mg/kg daily). This concentration was less than that reported by Wood *et al.*, who used 10 mg of T₃ per kg per day orally for six days, but greater than that following administration of T₃ orally or subcutaneously at $1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for six days.⁵ Dosing with microgram rather than milligram quantities of T₃ has been shown to sensitize the rat to halothane-induced hepatic necrosis, but the pretreatment period was appreciably longer. We have observed (unpublished studies) that rats anesthetized with halothane after pretreatment with T₃ at a dose of $10 \mu\text{g}$ per 100 g body weight per day intraperitoneally for 21 days developed hepatic centrilobular necrosis. Smith§§ found hepatic necrosis in rats given T₃ at a dose of $100 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ intraperitoneally for ten days and then anesthetized with halothane. By using milligram rather than microgram quantities of T₃, we shortened the pretreatment period. This allowed us to manipulate a large number of animals during a brief period, and simultaneously reduce the potential risk of intercurrent illness that can occur with a prolonged treatment schedule.

We found that the incidence and severity of hepatic centrilobular necrosis in T₃-treated rats were significantly greater at six hours after halothane than at six hours after isoflurane or enflurane anesthesia. The six-hour interval for removal of livers for histopathologic examination was selected because our previous study (unpublished results) showed that dosing the rat with T₃ at $5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for six days by the intraperitoneal route produced a greater incidence and severity of the lesion at six hours than removal of the livers

§§ Smith AC: Halothane-induced hepatotoxicity in hyperthyroid rats. Ph.D. Thesis, Vanderbilt University, 1982.

immediately, at two hours or at 24 hours after halothane anesthesia. This was in contrast to the previous study,⁵ which reported that hepatic necrosis was seen consistently immediately and at 24 hours after halothane anesthesia when rats were pretreated with oral or subcutaneous doses of T_3 .

Air was used as the carrier gas for isoflurane and enflurane to anesthetize T_3 -treated rats in order to compare the tendency of these anesthetics with that of halothane to induce the liver lesion. Wood *et al.*⁵ found that T_3 -pretreated rats developed hepatic necrosis following anesthesia with halothane in air and in 99% oxygen. Increasing the oxygen concentration lessened the necrosis, but did not prevent it. Vehicle-treated rats anesthetized with isoflurane in 12% oxygen did not develop hepatic necrosis, and in a previous study,⁵ no hepatic necrosis occurred in vehicle-treated rats anesthetized with halothane in 14% oxygen. However, there was a tendency for the liver lesion to be more severe in T_3 -treated rats anesthetized in 14% oxygen.⁵ These observations suggest that hypoxia during anesthesia increases the susceptibility of hypermetabolic liver cells to anesthetic-induced hepatotoxicity.

We found that enflurane induced hepatic necrosis in T_3 -pretreated rats, but Wood *et al.*⁵ did not observe a liver lesion in six T_3 -treated rats exposed to enflurane. Wood *et al.* pretreated their rats with 2 mg/kg of T_3 subcutaneously daily for five days and anesthetized the rats on the sixth day, and 24 hours later prepared the livers for histopathologic examination. In the present study, rats were pretreated daily for six days with 5 mg/kg of T_3 intraperitoneally, anesthetized on the seventh day, and the livers were removed six hours after anesthesia. Thus, the discrepancy between our results and those of Wood *et al.* may be explained by difference in dose, duration of treatment, route of administration of T_3 , and the time of removal of the livers for histopathologic examination. Furthermore, the low frequency of hepatic necrosis following enflurane exposure (40 T_3 -treated rats with two deaths during anesthesia and nine rats with liver lesion, a 24% incidence) suggests the lesion may not have been observed in as small a sample as six rats.

Halothane produced the greatest incidence of mortality in T_3 -treated rats during anesthesia. Although we do not know the mechanism of death, since we did not measure the requisite physiologic variables, it appears from the data (tables 3 and 4) that the incidence and severity of the hepatic lesion was unrelated to the incidence of death, at least, in those T_3 -treated animals anesthetized with isoflurane, enflurane, or pentobarbital. Liver necrosis occurred in T_3 -treated rats anesthetized with enflurane or isoflurane, but not in those

anesthetized with pentobarbital. By contrast, the mortality rate during pentobarbital anesthesia was significantly greater than during enflurane, and not different from that occurring during exposure to isoflurane.

The stresses we imposed on vehicle-treated rats, hypoxia during anesthesia with or without prior food deprivation, and food restriction to maintain the body weight gain similar to that of T_3 -treated rats, did not produce hepatic necrosis. Smith⁸ found no liver toxicity in halothane-anesthetized vehicle-treated rats rendered diabetic or deprived of food 48 hours preceding the anesthetic exposure in air. Furthermore, starvation in T_3 -treated rats prior to halothane exposure did not worsen the liver lesion, but had a protective effect.² These observations suggest that the stress of hypoxia, starvation, or diabetes individually does not sensitize the rat to anesthesia-induced hepatotoxicity.

It may be argued that differences in depth of anesthesia with the inhalational agents may have accounted for variations observed in the mortality and in the incidence and severity of the liver lesion. We used 1% halothane, because it has been shown previously that this concentration was effective in inducing the hepatic lesion in T_3 -treated rats,⁵ and 1% halothane was used to produce liver necrosis in the halothane hypoxic model.⁸ The concentrations of halothane and isoflurane we used were within 90 and 94%, respectively, of the MAC values reported for these agents in the rat,⁹ and suggest that the animals were exposed to approximately equipotent anesthetic concentrations of halothane and isoflurane. The concentration of enflurane, 1.8% was chosen empirically, because at this concentration of enflurane animals remained anesthetized during the period of exposure with minimal mortality. In a previous study (unpublished results), less than 10% of T_3 -treated and control rats survived two hours of exposure to 2% enflurane in air, and less than 25% of animals were anesthetized with 1% enflurane. We were unable to find a report for the MAC of enflurane in the rat, and do not know if the concentration of enflurane was equipotent with that of isoflurane and halothane. The observation that no hepatic lesion occurred in T_3 -treated pentobarbital-anesthetized rats, although the recovery from anesthesia with pentobarbital was more than 12 times longer than the recovery from halothane, isoflurane, or enflurane anesthesia, suggests that the inhalational agents *per se* and not the depth or duration of the anesthetized state effects the production of the liver lesion.

Liver injury by halothane is thought to be related to the biodegradation of halothane to reactive intermediates. This reasoning is based on observations made in the halothane hypoxic model.¹⁰⁻¹² In this model, hepatic

centrilobular necrosis occurs when phenobarbital-pretreated rats are exposed to halothane in the presence of low oxygen tensions. Under these conditions, halothane has been shown¹² to be metabolized to one or more reactive metabolites which covalently bind *in vitro* to liver proteins and phospholipids. Production of these reactive intermediates is inhibited by normal or high oxygen tensions. Thus, there appears to be a circumstantial link between halothane-induced liver toxicity and the reductive biotransformation of halothane to reactive intermediates. However, T₃ decreases liver cytochrome P-450⁵ which is opposite to the effect of phenobarbital which enhances the production of halothane intermediates. Isoflurane and enflurane are metabolized to very limited extents in oxygen and not at all in the presence of low oxygen tension.¹³ These observations suggest that, at least in T₃-treated rats, a mechanism other than formation of reactive intermediates may be involved in liver toxicity with isoflurane or enflurane. The central area of the liver lobule has been reported¹⁴ to be exquisitely sensitive to low oxygen tensions, undergoing necrosis at hypoxic levels that do not affect other regions of the liver. This observation, plus the observation that T₃ decreases liver cytochrome P-450 and that isoflurane and enflurane are relatively inert, leads us to speculate that anesthesia-related hepatotoxicity seen in T₃-treated rats may be hypoxic damage to hypermetabolic centrilobular cells resulting from isoflurane- and enflurane-induced depression of splanchnic blood flow. A similar mechanism also may account for the damage observed in T₃-treated rats following halothane anesthesia.¹⁵

The authors thank Donald P. Island, Assistant Professor of Medical Research and Technical Director of the Endocrine Laboratory, Vanderbilt University Hospital, for measuring the serum concentrations of triiodothyronine.

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