■ LABORATORY REPORT

Differential Effects of Volatile Anesthetics on Hepatic Heme Oxygenase–1 Expression in the Rat

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THE microsomal enzyme heme oxygenase (HO; EC 1.14.99.3) catalyzes the oxidation of heme to biliverdin-IXa, iron, and carbon monoxide.[1] So far, three isoforms of this enzyme have been cloned. While HO-2 and HO-3 are constitutively expressed,[2,3] HO-1 is highly inducible in response to a variety of stimuli and has been identified as the major 32-kd heat shock (stress) protein (HSP) 32.[4]

Heme oxygenase–1 is essential for the function of the normal liver and plays a major protective role in the stress-exposed liver.[5] It exerts antioxidant properties by cleavage of the prooxidant heme and by the production of biliverdin, which is subsequently reduced to the antioxidant bilirubin.[6] Furthermore, its product carbon monoxide serves to maintain liver perfusion[5] and has potent antiinflammatory effects.[7]

Many studies have shown differential effects of volatile anesthetics on the perfusion, function, and integrity of the liver. Moreover, preliminary evidence suggests that volatile anesthetics may interfere with stress gene expression and function.[8] We therefore aimed to determine whether volatile anesthetics affect hepatic HO-1 gene expression.

Materials and Methods

Animals

The experimental protocol was approved by the local animal care and use committee, and all animals received humane care according to the criteria outlined in the *Guide for the Care and Use of Laboratory Animals*.[9] Male Sprague-Dawley rats (Charles River, Sulzfeld, Germany) weighing 299 ± 16 g were subjected to arterial blood pressure monitoring *via* the carotid artery, and a tracheotomy was performed to maintain airway patency.[10] The animals were randomized into one of the following groups (n = 6 for each group): group 1 (control), anesthesia with 60 mg/kg pentobarbital sodium

intraperitoneally (Alvetra, Neumuenster, Germany) followed by intravenous bolus injections of 6 mg/kg every 30 min; group 2, isoflurane (2.3 vol%; Baxter, Unterschleissheim, Germany); group 3, desflurane (12 vol%; Baxter, Unterschleissheim, Germany); or group 4, sevoflurane (4 vol%; Abbott, Wiesbaden, Germany).

These concentrations of volatile anesthetics correspond to a minimum alveolar concentration in the rat of 1.6–1.8.[11-13] Volatile anesthetics were delivered in an atmosphere of 30% oxygen (Narcorex19; Draeger, Luebeck, Germany), continuously monitored (PM8050; Draeger, Luebeck, Germany), and spontaneously inhaled by the animals through a tracheotomy tube for the experiment's duration of 6 h. Body temperature was maintained at 38 ± 4°C. This regimen resulted in an arterial oxygen tension of 60–100 mmHg and an arterial carbon dioxide tension of 30–50 mmHg in all groups. Two animals that served as positive controls for the detection of HSP27 and HSP70 were subjected to 1 h of hemorrhagic shock and 5 h of resuscitation as previously described.[10] Additional experiments were performed to mimic volatile anesthetic-induced hypotension in rats anesthetized with pentobarbital using the vasodilator dihydralazine (n = 4; Novartis, Nuernberg, Germany). The dose of dihydralazine ranged from 4 to 8 mg · kg^{-1} · 6 h^{-1} and was adjusted to achieve a mean arterial pressure (MAP) of 76 ± 12 mmHg, which was the mean MAP of all animals subjected to volatile anesthetics.

RNA Extraction and Northern Blot Analysis

Total RNA was isolated from frozen liver tissue (TRIZOL; Gibco, Grand Island, NY) as previously described.[10] Ten micrograms of RNA were loaded per lane, size fractionated, and transferred to a nylon membrane. Hybridization was performed with P$^{32\gamma}$-deoxycytosine triphosphate–radiolabeled HO-1,[14] HSP27, and HSP70 complementary DNA.[10] All blots were reprobed with 18S ribosomal RNA complementary DNA to verify equal loading. Autoradiographs were analyzed by laser scanning densitometry (Personal Densitometer; Molecular Dynamics, Krefeld, Germany).

Western Blot Analysis

Frozen liver tissue was homogenized and lysed in TOTEX buffer (20 mM HEPES [pH 7.9], 0.35 M NaCl, 20% glycerol, 1% Nonidet P-40, 1 mM MgCl$_2$, 0.5 mM EDTA, 0.1 mM EGTA) as previously described.[10] Each lane of a
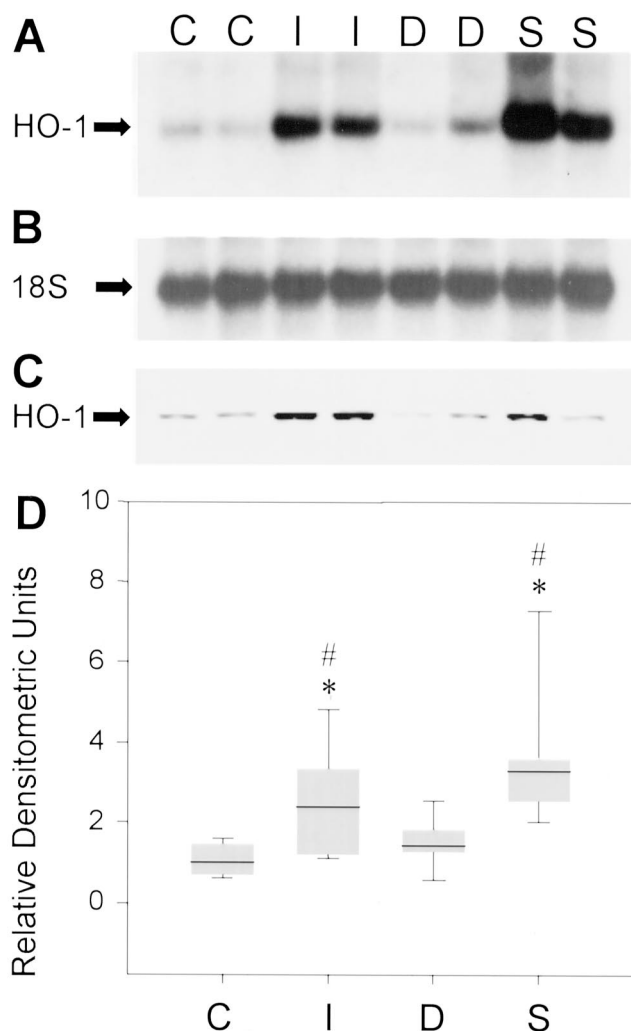
A

B

C

D

Fig. 1. Expression of heme oxygenase–1 (HO-1) messenger RNA (mRNA) (*A*), 18S ribosomal RNA (*B*), or HO-1 protein (*C*) in the livers of two representative animals subjected to 6 h of anesthesia with pentobarbital sodium (C), isoflurane (I; 2.3 vol%), desflurane (D; 12 vol%), or sevoflurane (S; 4 vol%). (*D*) Relative densitometric units of HO-1 mRNA concentrations. Data are presented as median (box: 25th and 75th percentiles; error bars: 10th and 90th percentiles) for n = 6 animals per group. *$P < 0.05$ *versus* pentobarbital control (C). #$P < 0.05$ *versus* desflurane (D).

12% sodium dodecyl sulphate polyacrylamide gel contained 100 μg of total protein. After protein separation and electroblotting, HO-1 was detected by a rabbit polyclonal anti–HO-1 antibody (1:1,000 dilution, SPA-895; StressGen Biotechnologies, Victoria, Canada) using the Enhanced Chemiluminescence (ECL) detection kit (Amersham Pharmacia, Freiburg, Germany) according to the manufacturer's instructions.

Data Analysis

Data are presented as median (box: 25th and 75th percentiles; error bars: 10th and 90th percentiles). Statistical differences between experimental groups were determined using a Kruskal–Wallis one-way analysis of

variance on ranks followed by a *post hoc* Student–Newman–Keuls test. A *P* value < 0.05 was considered to indicate a significant difference.

Results

Effect of Volatile Anesthetics on Heme Oxygenase–1 Gene Expression in the Liver

Hepatic HO-1 transcripts were barely detectable in the animals anesthetized with pentobarbital. In contrast, 6 h of anesthesia with isoflurane led to a substantial increase in HO-1 messenger RNA (mRNA) and protein (fig. 1). Densitometric analysis of all experiments revealed a more than 2.3-fold induction of HO-1 mRNA by isoflurane as compared to the pentobarbital control experiments (fig. 1).

Anesthesia with sevoflurane led to a 3.3-fold increase in HO-1 mRNA that tended to be even more pronounced as compared to isoflurane (fig. 1). In sharp contrast to the inducing effect of isoflurane and sevoflurane, desflurane did not upregulate HO-1—*i.e.*, the hepatic HO-1 mRNA content was comparable to that of pentobarbital controls (fig. 1). Western blot analyses revealed a similar pattern of HO-1 protein accumulation in response to the different anesthetic agents (fig. 1).

Effect of Volatile Anesthetics on Heat Shock Protein 27 and Heat Shock Protein 70 Gene Expression in the Liver

To test whether volatile anesthetics lead to a general induction of stress-inducible HSPs, the same RNA samples as shown in figure 1 were hybridized with radiolabeled HSP27 or HSP70 complementary DNA probes. No signal was detectable under control, isoflurane, desflurane, or sevoflurane anesthesia. To exclude the possibility that this was due to technical reasons, the RNA of two animals that had been subjected to hemorrhagic shock
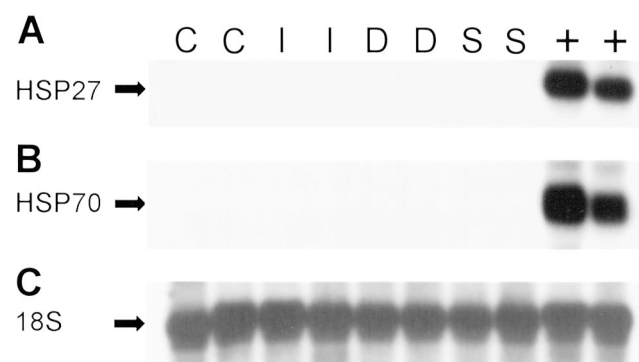
A

B

C

Fig. 2. Expression of heat shock protein (HSP) 27 messenger RNA (mRNA) (*A*), HSP70 mRNA (*B*), and 18S ribosomal RNA (*C*) in the livers of two representative animals subjected to 6 h of anesthesia with pentobarbital sodium (C), isoflurane (I; 2.3 vol%), desflurane (D; 12 vol%), or sevoflurane (S; 4 vol%). + = positive control.
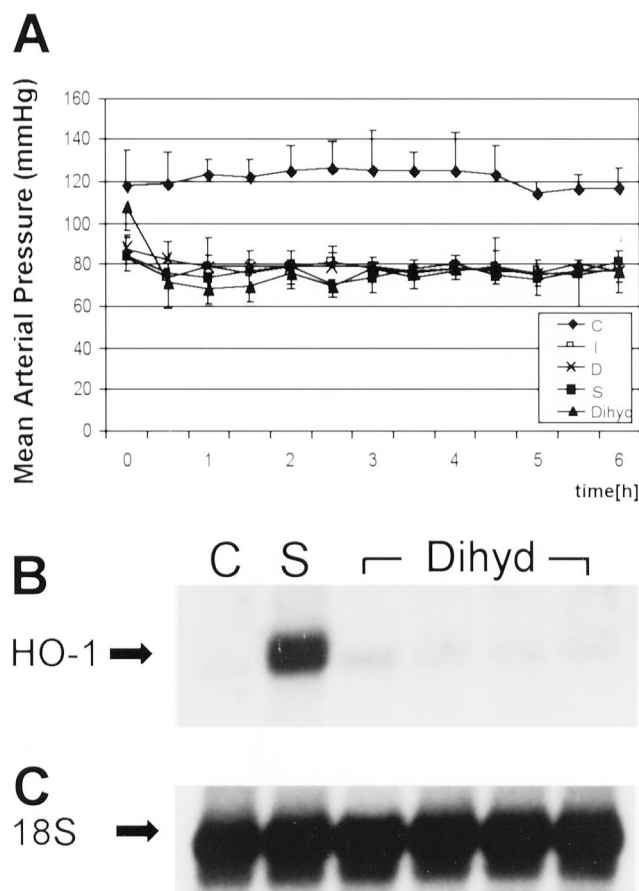
A

B

C   S   ⌐ Dihyd ⌐

HO-1 ➡

C

18S ➡

Fig. 3. (*A*) Mean arterial pressure of rats during anesthesia with pentobarbital sodium (C), isoflurane (I; 2.3 vol%), desflurane (D; 12 vol%), sevoflurane (S; 4 vol%), or pentobarbital + dihydralazine (Dihyd). Expression of heme oxygenase–1 (HO-1) messenger RNA (*B*) or 18S ribosomal RNA (*C*) in the livers of representative animals.

and resuscitation[10] were used as positive controls and showed a strong signal (fig. 2).

Effect of Hypotension on Hepatic Heme Oxygenase–1 Gene Expression

The MAP was approximately 35% lower in the isoflurane, sevoflurane, and desflurane groups as compared to the pentobarbital controls (fig. 3). To evaluate the role of hypotension in hepatic HO-1 induction, additional pentobarbital control experiments were performed using the vasodilator dihydralazine to lower the MAP to the same extent (76 ± 12 mmHg) as observed in the animals treated with isoflurane, desflurane, or sevoflurane (78 ± 8 mmHg). However, none of these animals exhibited HO-1 mRNA accumulation in the liver (fig. 3).

Discussion

Our aim in this study was to evaluate the effect of volatile anesthetics on hepatic HO-1 expression. Our results indicate that different volatile anesthetics differ-

entially regulate HO-1 expression in the normal liver. Whereas isoflurane and sevoflurane induced HO-1 mRNA and protein, desflurane did not affect the expression of this gene.

It has been shown previously that halothane may cause hepatic expression of HO-1 in rats pretreated with phenobarbital if administered under hypoxic conditions.[15,16] In contrast, neither halothane nor isoflurane induced HO-1 in the liver during hypoxia in the absence of phenobarbital pretreatment in these studies.[16] This discrepancy between the historical data and the observations described here is most likely due to the fact that the total amount of volatile anesthetics administered was much lower in the former study. While Yamasaki *et al.*[16] used a 0.3 minimum alveolar concentration of isoflurane, our experimental protocol included the administration of a 1.7 minimum alveolar concentration. Moreover, the time of exposure to the volatile anesthetics was limited to 2 h in the previous reports, while it was 6 h in the present study. Therefore, the sum of these data would be consistent with a dose and time dependency of the inducing effect of volatile anesthetics on hepatic HO-1 expression.

The reason for the differential induction of HO-1 by different volatile anesthetics is not known. However, it could be related to differences in the degree of hepatic cellular stress after exposure to these agents.[17–19] Thus, we determined the effect of the different volatile anesthetics on the expression of other stress-inducible heat shock proteins, such as HSP27 and HSP70, under the same experimental conditions.[10,15] However, neither HSP27 nor HSP70 mRNA could be detected after 6 h of anesthesia with the various agents. This is in accordance with previous reports that have demonstrated induction of HSP70 by volatile anesthetics only in combination with phenobarbital pretreatment and hypoxia but not by volatile anesthetics alone.[15,16,20,21] These findings suggest that the signal transduction pathway responsible for volatile anesthetic-mediated HO-1 expression is independent of the one leading to the induction of the general heat shock response.

To exclude the possibility that the reduction in arterial blood pressure by volatile anesthetics might be involved in HO-1 induction, we lowered the arterial blood pressure in additional animals anesthetized with pentobarbital to a similar extent as in the volatile anesthetic groups. However, no induction of HO-1 could be observed under these conditions. Therefore, hypotension itself does not seem to be responsible for HO-1 induction. Moreover, since isoflurane, sevoflurane, and desflurane decreased the MAP to the same degree, this cannot explain their differential effects on HO-1 expression.

Taken together, our findings demonstrate that volatile anesthetics differentially induce HO-1 mRNA in the liver, and this effect does not extend to other HSPs. Therefore, volatile anesthetics can be considered as specific gene

regulators of hepatic HO-1, an enzyme that may exert potent organ protective functions.[5]

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