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Effect of Halothane on Synthesis and Secretion of Liver Proteins

KATHRYN E. FLAIM, LEONARD S. JEFFERSON, JOAN B. MCGWIRE, AND D. EUGENE RANNELS[1]

Departments of Physiology and Anesthesia, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania 17033

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SUMMARY

The effect of halothane on synthesis of retained and secreted proteins was investigated using isolated perfused rat livers. Anesthetic exposure rapidly inhibited synthesis of total liver proteins in a dose-dependent manner by a mechanism which appeared to involve reduced rates of both peptide chain initiation and elongation. While halothane concentrations comparable to the clinical dose resulted in small changes in protein metabolism, higher concentrations (4%) of the anesthetic had marked effects. At early time points, relative rates of albumin synthesis were unaffected by halothane, but, as anesthetic exposure was prolonged, production of albumin and of total secreted plasma proteins was inhibited more extensively than that of retained liver proteins. Thus, halothane appeared to exert differential inhibitory effects on synthesis of these two classes of liver proteins.

INTRODUCTION

Previous studies have established that anesthetics exert a variety of metabolic effects in a number of tissues, including liver and lung. These alterations in cellular metabolism include inhibition of the rate of incorporation of radioactive amino acids into protein (1, 2). The most detailed information regarding this effect is derived from studies of isolated lung tissue (3, 4) and lung cells (5), where halothane exposure led to a rapid, dose-dependent inhibition of total protein synthesis exerted at the cellular level. This reversible inhibition did not involve extensive depletion of cellular energy stores or nonspecific changes in membrane permeability, nor did it reflect effects of the anesthetic on the specific radioactivity of the pool of amino acids serving as precursors to the synthetic pathway (4).

The mechanism of action of anesthetic hydrocarbons is thought to involve hydrophobic interactions of these agents at the membrane level. In this regard, it could be postulated that synthesis of secretory proteins on membrane-bound polyribosomes might be inhibited preferentially in anesthetic-exposed tissues. For example, local anesthetics, which also exhibit interactions with membranes, impair production of collagen (6).

The isolated perfused rat liver provides an ideal model system for investigation of this hypothesis. Extensive information is available regarding the physiological regulation of synthesis of liver proteins (7); many regulatory variables can thus be controlled *in vitro*. Futhermore, a major portion of newly synthesized liver proteins is secreted (30%), with serum albumin accounting for most of this fraction (8). Thus, the synthesis and secretion of serum albumin as a model secretory protein can be monitored by using immunochemical methods (8, 9).

METHODS

Male Sprague-Dawley rats [Crl:CD (SD) BR] (Charles River Breeding Laboratories, Wilmington, Mass.) were maintained on a 12-hr light/12 hr dark cycle and were provided food (Charles River diet RMH 3000) and water ad libitum. Animals (110–130 g) were anesthetized with an i.p. injection of sodium pentobarbital (8 mg/100 g of body weight; Fort Dodge Laboratories, Fort Dodge, Ind.) and were prepared for *in situ* liver perfusion as has been previously described (9, 10). Briefly stated, livers were perfused at a flow rate of 7 ml/min with a medium consisting of Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 10 mM glucose, 3% (w/v) bovine serum albumin (Fraction V, Miles Laboratories, Elkart, Ind.), 29% washed bovine erythrocytes, and 19 amino acids at 5 times rat arterial plasma concentrations (11). Leucine was added to the perfusate plasma at a concentration of 5 mM, and L-[4,5-^{3}H]leucine was added as indicated below for determination of rates of protein synthesis. Previous studies have shown that, under these conditions, the specific radiaoctivity of leucyl-tRNA is equal to that of extracellular leucine in 2 min (10). The perfusion medium was maintained at 37° and was gassed with a mixture of 95% O_2 and 5% CO_2. At the times indicated in table and figure legends, halothane (2-bromo-2-chloro-1,1,1-trifluorethane; Fluothane, Ayerst Laboratories, New York, N. Y.) was mixed with the gas by using a Fluotec 3 vaporizer (Fraser, Sweatman, Lancaster, N. Y.) such that the perfusate was equilibrated with 2% or 4% halothane.

For determination of rates of total liver protein synthesis and relative rates of albumin synthesis, livers were perfused in a non-recirculating manner, and medium containing [^{3}H]leucine (5.33 μCi/ml of perfusate plasma; specific radioactivity, 2345 dpm/μmole) was delivered for 15 min following a period of preperfusion with nonradioactive perfusate. Samples of perfusate and liver were then taken for determination of perfusate leucine specific radiaoctivity and incorporation of radioactivity into trichloroacetic acid-precipitable liver protein, as has been previously described (10, 12). Protein determinations were made using

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the biuret method (13). Rates of protein synthesis are expressed as milligrams of protein synthesized per gram of liver per hour (10). Relative rates of albumin synthesis were determined on samples of frozen liver homogenized in 3 volumes of 0.25 M sucrose containing Triton X-100 and sodium deoxycholate (1%), by trichloroacetic acid precipitation of total protein on fiber discs and immunoprecipitation of albumin with a specific anti-rat albumin antibody as described previously (8, 9). Incorporation of radioactivity into albumin is expressed as a percentage of the radioactivity incorporated into total protein.

In order to determine rates of secretion of albumin and total secretory protein [^{3}H]leucine (6.7 μCi/ml of perfusate plasma) was added to 100 ml of recirculating perfusate after a preperfusion of 30 min. Perfusion was then continued for an additional 2 hr, and samples of medium were taken at 10, 40, 60, 80, 100, and 120 min of this labeling interval. Erythrocytes were removed by centrifugation, and the leucine specific radioactivity was determined on the trichloroacetic acid-soluble fraction of the plasma. Aliquots of plasma were also analyzed for radioactivity in acid-precipitable total protein and immunoprecipitable albumin (8, 9). Livers were removed at the end of the labeling period for determination of liver weight and of incorporation of radioactivity into nonsecreted liver protein by acid precipitation of frozen liver powder as described earlier (10, 12).

In some experiments in which rates of total protein synthesis were measured, sampels of liver were also taken for determination of the relative concentrations of RNA in ribosomal subunits. Livers were homogenized in a Dounce homogenizer in 7 volumes of buffer [250 mM KCl, 2 mM magnesium acetate, 250 mM sucrose, and 20 mM Tris-HCl (pH 7.4)]. The 10,000 × *g* supernatant of the preparation (0.75 ml) was layered on 0.44–2.0 M exponential sucrose gradients made in the above buffer and was centrifuged in an SW 41 rotor (Beckman Instruments) at 37,000 rpm for 18 hr to achieve an $\omega^2 t$ of 9.79×10^{11}. The A_{254} of the gradients was monitored using a density gradient fractionator (Instrument Specialties Company, Lincoln, Nebr.), and ribosomal subunit and monomer fractions were collected. RNA contents of the gradient fractions and of the homogenate were determined by alkaline hydrolysis (14).

Sample means were compared using a one-way analysis of variance followed by the Student-Newman-Keuls multiple-range test. Curve fitting was performed using linear regression analysis by the least-squares method. A p value of less than 0.05 was considered to be significant.

RESULTS

Rates of total protein synthesis were determined over a 15-min interval following a 30-min exposure to 4% halothane (Table 1). The use of a 15-min labeling period ensured that incorporation of radioactivity into all classes of liver protein was measured, because newly synthesized secretory proteins remain in liver for at least this period of time (9, 15). Relatively brief exposure to halothane resulted in a 20% decrease in the over-all rate of liver protein synthesis.

The nature of this inhibition of protein synthesis was further investigated by determining the relative numbers of ribosomal particles existing as free subunits (Fig. 1). Under normal conditions, peptide chain elongation is rate-limiting to the protein synthetic pathway, and most ribosomal material exists as polyribosomes. With the development of a block in peptide chain initiation, polysomes become disaggregated, and ribosomal subunits accumulate as free particles. As seen in these representative profiles, halothane appears to have caused a small increase in free subunits; however, this effect was variable. In one set of experiments a 25% increase in subunit RNA levels was statistically significant, whereas in the other no significant increase in subunit levels occurred.

TABLE 1

Effect of halothane exposure on rates of total protein synthesis and relative rates of albumin synthesis in perfused liver

Livers were preperfused for 15 or 105 min with medium containing 5 mM nonradioactive leucine. Synthesis of proteins was estimated during a subsequent 15-min interval by addition of 5 mM [^{3}H]leucine. In livers exposed to halothane, the anesthetic was introduced an average of 15 min prior to the start of the preperfusion. At the end of the experiment, livers were taken for determination of rates of total protein synthesis and albumin synthesis relative to total as described in the text. Values are means ± standard error of the mean of six to eight determinations.

Parameter and interval	Control	2% Halothane	4% Halothane
Total protein synthesis (mg/g liver·h^{-1})			
15–30 min	3.57 ± 0.05	ND[a]	2.90 ± 0.11[b]
105–120 min	3.78 ± 0.17	3.05 ± 0.18[b]	2.79 ± 0.25[b]
Relative rate of albumin synthesis (% total)			
15–30 min	11.8 ± 0.4	ND	11.4 ± 0.3
105–120 min	12.1 ± 0.3	11.8 ± 0.7	9.4 ± 1.1[b]

[a] ND, Not determined.
[b] $p < 0.05$ versus control.

If the inhibition of total protein synthesis were solely attributable to a block at the level of peptide chain initiation, a more clear indication of polysomal disaggregation would be expected (16). Rather, the significant inhibition of protein synthesis with little or no concomitant polysomal disaggregation suggests that both initiation and elongation reactions were impaired. Thus, subunits were not released as rapidly from polyribosomes and did not accumulate as free particles. The results of the present study suggest that a generalized slowing of the entire translational process occurs in the presence of halothane.

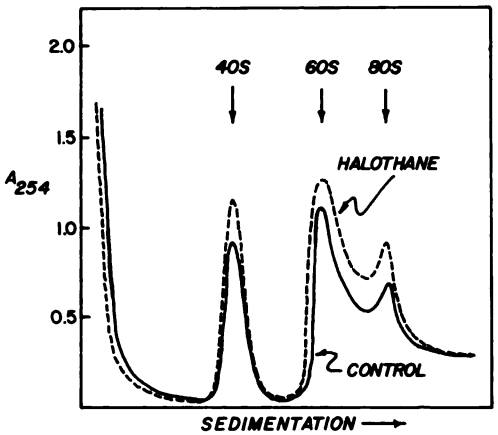

FIG. 1. *Effect of halothane exposure on A_{254} profiles of ribosomal subunits isolated on sucrose density gradients*

Livers were perfused for 45 min in the presence of a normal gas mixture (control) or 4% halothane as indicated in Table 1 and in the text. Livers were homogenized, the postmitochondrial supernatants were layered on 0.44–2.0 M sucrose density gradients, and the gradients were centrifuged at 37,000 rpm for 18 hr in an SW 41 rotor. Gradients were pumped through a flow cell, and A_{254} was monitored. The profiles shown are representative of eight profiles for each condition.

When the duration of exposure to 4% halothane was extended to 2 hr, total protein synthesis declined slightly, although not significantly, to 74% of the control rate (Table 1). Although prolonged exposure to the anesthetic did not lead to increased inhibition, the extent of inhibition was dose-related; 2% halothane resulted in an intermediate (18%) reduction in total protein synthesis.

As proposed in the introductory statements, the inhibitory action of halothane may not affect synthesis of all classes of proteins equally, but may primarily involve synthesis of secretory proteins on membrane-bound polyribosomes. However, since 30% of liver protein synthesis is directed toward the synthesis of secreted proteins, a nearly complete inhibition of the production of secreted proteins would be required to account for the entire decline in total protein synthesis measured above (4% halothane). As an indication of the extent of inhibition of total secretory protein synthesis, the relative rate of synthesis of the most abundant secretory protein, albumin, was determined in these same liver samples. As seen in Table 1, in the short-duration studies there was no suggestion of a selective alteration in the synthesis of albumin upon exposure of the livers to 4% halothane. After 2 hr, however, relative rates of albumin synthesis were decreased by 23%, suggesting that a preferential inhibition of the synthesis of secretory proteins developed as a function of time in livers exposed to 4% halothane. In parallel experiments, 2% halothane did not reduce the relative rate of albumin synthesis to a significant extent (14%; Table 1).

The observation that halothane may alter secretory protein production selectively was tested more directly in perfusion experiments in which the incorporation of radioactivity into albumin and total plasma protein appearing in the perfusate was monitored over a 2-hr interval (Fig. 2). The production of both total plasma protein

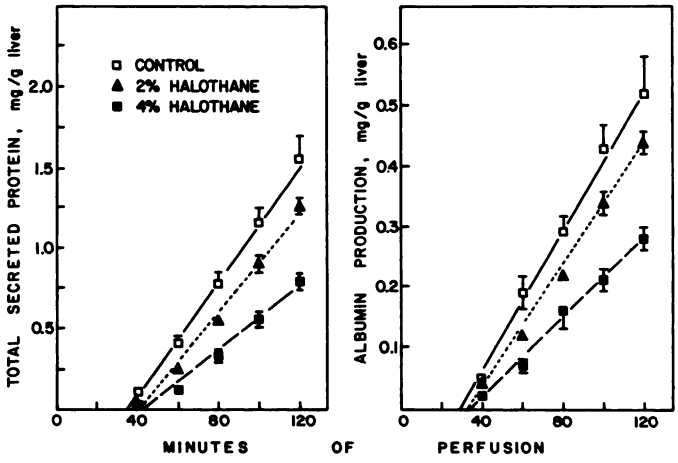

FIG. 2. *Effect of halothane exposure on the production of total secretory protein and albumin in perfused liver*

Livers were preperfused for 30 min with 5 mM leucine, and 5 mM [^{3}H]leucine was introduced for an additional 2 hr. Perfusate samples were collected and assayed for incorporation of radioactivity in total secretory protein and albumin as described in the text. Halothane exposure was initiated 15 min after the start of the experiment. Points indicated means ± standard error of the mean of six to eight determinations. Lines were drawn by linear regression analysis of all points in each experimental condition.

TABLE 2

Effect of halothane exposure on the production of secretory proteins by perfused liver

Livers were perfused as described in the text and in Fig. 2. Perfusion media were sampled and assayed for incorporation of radioactivity into total secretory protein and albumin as indicated in the text for determination of secretory protein synthesis and secretion times. Values are means ± standard error of the mean of six to eight observations.

Parameter	Total secretory Protein	Albumin
Protein synthesis (mg/g liver·hr^{-1})		
Control	1.09 ± 0.08	0.35 ± 0.03
Halothane		
2%	0.92 ± 0.04	0.31 ± 0.01
4%	0.61 ± 0.05[a]	0.20 ± 0.01[a]
Secretion time (min)		
Control	37 ± 1	30 ± 2
Halothane		
2%	41 ± 2[a]	34 ± 1
4%	45 ± 2[a]	36 ± 3

[a] $p < 0.05$ versus control.

and albumin was affected by halothane in a dose-dependent manner. The higher anesthetic dose (4%) decreased rates of secretion (Fig. 2; Table 2) by 44% in the case of total plasma protein and by 43% in the case of albumin. An intermediate, but not statistically significant, decline in protein production was evident in livers exposed to 2% halothane. The delay from the onset of labeling until radioactively labeled albumin appeared in the medium—the secretion time—was not markedly altered by halothane. In contrast, the secretion time for toal plasma protein was increased to a small but significant extent at both anesthetic doses.

DISCUSSION

Hydrophobic interactions of anesthetic hydrocarbons at the membrane level appear to be linked to their mechanism of action (17). In this context, halothane alters a number of cellular membrane functions, including amine transport (18, 19) and receptor-ligand interactions (20). Recent studies of the allosteric effects of volatile anesthetics on the binding kinetics of α-bungarotoxin to the membrane-bound acetylcholine receptor suggest strongly that these agents may cause specific rather than general alterations in membrane structure or function (20, 21). These conclusions are consistent with the present observations that the secretion time of newly synthesized plasma proteins was little affected in livers exposed to high doses of halothane. Furthermore, they support the conclusion drawn in earlier studies of lung (4, 19) that gross changes in membrane integrity are not involved in the inhibition of protein synthesis in halothane-exposed tissues.

The studies shown in Table 1 and Fig. 2 support the conclusion that, after prolonged exposure of liver to 4% halothane, the reduced production of secretory proteins reflects preferential inhibition of their synthesis with little effect on the secretion process itself. In the 2-hr experiments, total protein synthesis was reduced 26%. An additional reduction of the relative rate of albumin syn-

thesis from 12.1% to 9.4% of total protein predicts that, with no change in secretion, albumin production would be reduced 43%, a value which agrees exactly with that observed when the appearance of newly synthesized albumin was monitored in the perfusate. In contrast to the present study, in cultured 3T3 cells and human fibroblasts, local anesthetics appeared to decrease collagen secretion independent of inhibition of total protein synthesis (6). These observations imply that in lung, liver, or other tissues, although the effects of the anesthetic on membranes and inhibition of protein synthesis may coincide, they are not necessarily linked in a causal relationship.

In previous studies of the effects of halothane on synthsis of lung proteins, the magnitude of inhibition was linearly related to anesthetic dose (3–5). The present experiments confirm those observations. Whereas halothane concentrations comparable to those used clinically resulted in marginal changes in protein synthesis, higher concentrations of the anesthetic had marked effects. Therefore, it is possible that clinical levels of halothane in combination with other factors which inhibit protein synthesis, such as amino acid deprivation (10) or exposure to other anesthetics (22), could lead to a significant inhibition. In contrast to the progressive effects of halothane on amine transport in isolated lungs (19), the inhibition of total protein synthesis did not grow larger with time.

The underlying signal mediating the inhibitory effect of halothane is, as yet, undetermined, although certain possibilities can be considered. For example, administration of halothane to perfused liver preparations interferes with ATP production (23); furthermore, liver could be protected from this effect of halothane by the presence of fatty acids in the medium (24). These findings suggest that in the present study the protein synthetic process may have been inhibited because of a limitation of energy substrates, although two observations argue against this possibility. In these studies, livers were provided excess amino acids which should, as oxidizable substrates, have provided the same kind of protection as did fatty acids in the earlier study. Furthermore, in intact lung and isolated lung cells exposed to the anesthetic under similar conditions, halothane did not influence tissue ATP content, although the extent of inhibition of protein synthesis was comparable to that observed in liver (3–5).

Another possibility is suggested by the demonstrated effect of halothane on membrane transport functions (18, 19). An inhibition of amino acid transport could cause an intracellular amino acid deficiency which would result in reduced rates of protein synthesis (10). This, too, seems unlikely under the present circumstances. First, studies in perfused lung indicated that halothane does not alter amino acid transport or lower intracellular amino acid concentration (3, 4). Second, in the present study, not only were amino acids present in excess, but the previously demonstrated effect of amino acid deficiency on liver protein synthesis involves a potent inhibition of peptide chain initiation reactions and, therefore, differs in fundamental characteristics from the effect of halothane (16).

In summary, exposure of the perfused liver to halothane elicited a rapid and dose-dependent inhibition of total protein synthesis. After exposure of 2-hr duration, the inhibition of secretory protein production was more pronounced than that of total protein synthesis. The appearance of these proteins in the perfusate was reduced by more than 40% as compared with control values, reflecting an inhibition of their synthesis rather than a step in the secretory process.

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Send reprint requests to: Dr. D. Eugene Rannels, Department of Physiology, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pa. 17033.