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Reversible inhibition of protein synthesis in lung by halothane

D. Eugene RANNELS,* Rose CHRISTOPHERSON and Clyde A. WATKINS
Departments of Physiology and Anesthesia, Milton S. Hershey Medical Center, Pennsylvania State University, Hershey, PA 17033, U.S.A.

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Alterations in the synthesis and degradation of proteins were investigated in intact lungs exposed to the volatile anaesthetic halothane. In rat lungs perfused *in situ* with Krebs–Henseleit bicarbonate buffer containing 4.5% (w/v) bovine serum albumin, 5.6 mM-glucose, plasma concentrations of 19 amino acids and 690 μM-[U-^{14}C]-phenylalanine and equilibrated with $O_2/N_2/CO_2$ (4:15:1), protein synthesis, calculated based on the specific radioactivity of aminoacyl-tRNA, was inhibited by halothane. The anaesthetic did not affect degradation of lung proteins. The inhibition of protein synthesis was rapid in onset, dose-dependent, and quickly reversible. It did not appear to be associated with overall energy depletion, with non-specific changes in cellular permeability, or with decreased availability of amino acids as substrates for protein synthesis.

Halothane and other anaesthetic agents are well known to affect a number of metabolic pathways, including those of oxidative, glycolytic and protein metabolism. Halothane exposure produced a dose-related inhibition of glutamate oxidation by mitochondria isolated from rat liver, as well as an inhibition of oxygen uptake in several tissues (Cohen, 1973). These general effects may have been linked to stimulation of glycolysis in livers from fed rats and to inhibition of gluconeogenesis and urea synthesis in perfused livers from starved animals (Biebuyck *et al.*, 1972*a*,*b*). Overnight exposure of cultured lymphocytes to halothane inhibited the increase in [^{14}C]leucine incorporation that normally follows phytohaemagglutinin treatment (Bruce, 1975). Furthermore, metabolic effects of anaesthetic agents may be enhanced when several drugs are administered in combination (Rannels *et al.*, 1982*a*) or when tissue function is otherwise impaired, for example by hypoxia (McLain *et al.*, 1979; Ross *et al.*, 1979). Both N_2O (Nahrwold & Cohen, 1973) and steroid administration (Wozniak, 1978) increased the inhibitory effect of halothane on mitochondrial respiration.

Most studies of the metabolic effects of halothane have been performed in liver, reflecting clinical concern about the potential hepatotoxic effects of the drug or its metabolites (Cohen, 1977). In contrast, although the agent is introduced through, and thus reaches its highest concentration in, the respiratory system, almost nothing is known about the effects of halothane on lung metabolism. Several studies reported inhibitory effects of halothane on uptake and metabolism of amines from the pulmonary circulation. Clinical doses of halothane decreased the uptake of circulating noradrenaline by lungs of intact dogs (Bakhle & Block, 1976) and by perfused rabbit lungs (Naito & Gillis, 1973), and decreased uptake of 5-hydroxytryptamine by perfused rat lungs (Watkins *et al.*, 1983). Effects of the anaesthetic on carbohydrate or lipid metabolism have not been studied in lung tissue. Hammer & Rannels (1981) demonstrated that exposure of rabbit pulmonary macrophages to halothane rapidly inhibited protein synthesis in a dose-dependent and reversible manner. A similar inhibition was reported in preliminary studies with intact perfused rat lungs, where the effect of the drug was also exerted at the cellular level (Wartell *et al.*, 1981). The present studies were designed to confirm and extend the latter observations and to investigate the potential role of changes in amino acid availability in the mechanism of the halothane-induced inhibition of protein synthesis.

* To whom reprint requests should be addressed (Department of Physiology).

Materials and methods

Perfusion of rat lungs in situ

Male Sprague–Dawley rats were purchased from Charles River Laboratories (Wilmington, MA, U.S.A.) and were given Agway RMH 3000 chow and water *ad libitum*. The animals were housed in a

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12h-light/12h-dark cycle. Rats were anaesthetized with an intraperitoneal injection of sodium pentobarbital (50mg/kg body wt), weighed and prepared for lung perfusion *in situ* as detailed previously (Watkins & Rannels, 1979). Perfusion parameters were as follows: tidal volume, 1.0ml/100g body wt.; respiratory rate, 72breaths/min; positive end expiratory pressure, 2cmH_2O; pulmonary arterial pressure, 20cmH_2O; pulmonary venous pressure, 0cmH_2O; temperature, 37°C.

Unless otherwise stated, the perfusion buffer was a modified Krebs–Henseleit bicarbonate buffer (Rannels *et al.*, 1979) containing 5.6mM-glucose, 4.5% (w/v) bovine serum albumin (fraction V, Pentex; Miles Laboratories), 19 amino acids at concentrations measured in rat plasma (Watkins & Rannels, 1980) and radioactive phenylalanine (690μM). High concentrations of phenylalanine (10 times those found in plasma) were used to minimize dilution of the specific radioactivity of the amino acid with non-radioactive phenylalanine released from protein degradation. Increasing extracellular phenylalanine to this concentration did not affect the rate of protein synthesis (Watkins & Rannels, 1980). In all experiments, the lungs were ventilated and the perfusate was equilibrated with warmed (37°C) humidified $O_2/N_2/CO_2$ (4:15:1).

Exposure of the lungs to halothane

Where indicated, the gas mixture above was equilibrated with halothane (2-bromo-2-chloro-1,1,1-trifluoroethane; Fluothane; Ayerst Laboratories, New York, NY, U.S.A.) by directing the gas flow to the buffer reservoir and ventilator (>4 litres/min) through a Fluotec 3 vaporizer (Fraser Sweatman, Lancaster, NY, U.S.A.). Halothane concentrations in the perfusion medium were confirmed by using a Hewlett–Packard 5840-A gas–liquid chromatograph standardized against halothane solutions of known concentration. For these calibrations, an aqueous solution in equilibrium with a gas phase containing 4% halothane was calculated to contain the anaesthetic at a concentration of 1.09mM. Separation of halothane, on a Chromosorb 102 column, was performed exactly as detailed by Hammer & Rannels (1981).

Measurements of protein synthesis and degradation

For estimates of protein synthesis, perfused lungs were rinsed in 0.15M-NaCl (4°C), trimmed free of large airways, blotted on filter paper, and homogenized in 2.0ml of 0.5M-$HClO_4$. The acid-insoluble fraction was washed for determination of the incorporation of radioactive amino acid into lung proteins as described previously (Rannels *et al.*, 1979; Watkins & Rannels, 1980). Rates of protein synthesis (nmol of phenylalanine incorporated/h per mg of protein) were calculated by dividing incorporation (d.p.m./h per mg of protein) values by the specific radioactivity (d.p.m./nmol) of phenylalanine in the extracellular or aminoacyl-tRNA pool, as indicated. Previous studies (Watkins & Rannels, 1980) showed that, when extracellular phenylalanine was present at high concentration, the specific radioactivity of the amino acid in these pools rapidly reached a steady state and remained constant throughout at least 3h of perfusion.

Protein degradation was estimated by measuring release of phenylalanine into perfusion buffer which was devoid of phenylalanine and contained 20μM-cycloheximide to inhibit protein synthesis (Rannels *et al.*, 1975). Because phenylalanine was not metabolized by perfused lungs, except in the pathways of protein synthesis and degradation (Watkins & Rannels, 1980), net changes in the concentration of the free amino acid reflected the balance between these pathways. In the presence of cycloheximide, net release of phenylalanine provided a direct index of the rate of proteolysis. In these studies, perfusion buffer volumes were measured at the end of the experimental period by isotope dilution.

Measurements of amino acid content and specific radioactivity in lung tissue and perfusate

For amino acid analysis, lungs were trimmed and blotted as described above, and homogenized in 0.5M-$HClO_4$ in a Polytron PT-10 (Brinkman) homogenizer. Perfusate samples were precipitated with an equal volume of 1.0M-$HClO_4$. Extracts of tissue or perfusate were neutralized with KOH, the soluble fraction was freeze-dried, and the residue was taken up in 0.05M-lithium citrate buffer, pH2.2, and analysed with a Beckman 119Cl amino acid analyser, as described by Rannels *et al.* (1975). Radioactivity was determined by using a portion of the citrate-buffer sample and was assumed to be present only in phenylalanine, as shown by previous studies (Watkins & Rannels, 1980). This assumption was validated periodically. Intracellular amino acid concentrations and the specific radioactivity of phenylalanine were calculated as detailed previously (Watkins & Rannels, 1980).

For measurements of the specific radioactivity of phenylalanyl-tRNA, perfused lungs were frozen at liquid-N_2 temperatures and extracted exactly as described by Watkins & Rannels (1980). After deacylation of aminoacyl-tRNA, the dried residue of the fraction containing free amino acids was taken up in 100μl of 0.1M-$NaHCO_3$, pH9.8, and mixed with an equal volume of acetone containing 2mM-[^{14}C]dansyl (5-dimethylaminonaphthalene-1-sulphonyl) chloride (Amersham/Searle) of known specific radioactivity. Dansyl-amino acid derivatives were separated by t.l.c., and the specific radioactivity of [^{3}H]phenylalanine was calculated

from the radioactivity in the dansyl-phenylalanine spot (McKee *et al.*, 1978; Watkins & Rannels, 1980). Perfusate samples were analysed similarly.

Other analyses

Tissue contents of ATP and sorbitol or amino acid spaces were determined as described previously (Watkins & Rannels, 1979). Verapamil used in these studies was supplied by Knoll Pharmaceutical Co. Diltiazem was provided by Marion Laboratories. Significance of differences between means was determined by using Student's *t* test; *P* values <0.05 were considered to be significant.

Results

Effect of halothane on the synthesis of lung proteins

When rat lungs were perfused *in situ* with Krebs–Henseleit bicarbonate buffer equilibrated with $O_2/N_2/CO_2$ (4:15:1), incorporation of [^{14}C]-phenylalanine into protein began without a lag and continued at a linear rate for at least 3h (Table 1). Equilibration of the same gas mixture with halothane vapour led to a dose-dependent inhibition of phenylalanine incorporation. The effect of the anaesthetic appeared to be rapid in onset; a significant (25%) inhibition of phenylalanine incorporation was observed as early as 15min after exposure of the lungs to 4% halothane (Table 1).

In preliminary studies (Wartell *et al.*, 1981), the inhibition of phenylalanine incorporation during halothane exposure was assumed to reflect inhibition of protein synthesis by the anaesthetic. Quantitative measurements of the rate of protein synthesis require, however, that the specific radioactivity of the immediate precursor to protein, aminoacyl-tRNA, be taken into account (for reviews, see Rannels *et al.*, 1977, 1982*b*), under both control and experimental conditions. Previous studies showed that, under control conditions, increasing the extracellular phenylalanine concentration to 690μM led to a specific radioactivity of phenylalanyl-tRNA that did not differ significantly from that of phenylalanine in the perfusion medium (Watkins & Rannels, 1980). The present results confirmed that, when [^{3}H]phenylalanine was added to the perfusate at high concentration, rates of protein synthesis calculated from the specific radioactivity of extracellular or tRNA-bound phenylalanine did not differ significantly (Table 2). Rates of synthesis calculated in these lungs agreed closely with those determined previously (Watkins & Rannels, 1980). Similarly, in lungs exposed to 4% halothane, the specific radioactivity of phenylalanyl-tRNA reached 101% of that of the amino acid in the extracellular pool (Table 2). These observations showed that exposure of the lungs to halothane, even at high doses, did not modify the specific radioactivity of the pool of immediate precursor for the protein-synthetic pathway and that the extent of inhibition (46%) of protein synthesis by 4% halothane was reflected accurately when calculations were based on the specific radioactivity of extracellular phenylalanine. The rapid onset of the inhibition of phenylalanine incorporation by halothane, as well as the linearity with respect to time of amino acid incorporation in the presence of the drug (Table 1; Wartell *et al.*, 1981), suggested that in anaesthetic-exposed lungs the specific radioactivity of phenylalanyl-tRNA rapidly reached a steady state, as it did in control tissues (Watkins & Rannels, 1980).

From these observations, rates of protein synthesis were calculated from the data in Table 1 by linear regression analysis of phenylalanine incorporation and by using the specific radioactivity of extracellular phenylalanine. These calculations showed that synthesis of lung proteins was inhibited by halothane in a fashion linearly related to the dose

Table 1. *Effect of halothane on incorporation of* [^{14}C]*phenylalanine into lung protein*

Lungs were perfused for the period indicated as described in the Materials and methods section. The perfusate contained 690μM-[^{14}C]phenylalanine (sp. radioactivity 320d.p.m./nmol) and was equilibrated with halothane, as indicated. Data represent means ± S.E.M. for the numbers of observations shown in parentheses. Abbreviation: N.D., not determined. *$P<0.05$ and **$P<0.01$ versus control, without halothane.

Halothane (%)	[^{14}C]Phenylalanine incorporation (d.p.m./mg of protein)				
Time of perfusion (min) ...	15	30	60	120	180
0	157 ± 13 (11)	298 ± 19 (11)	557 ± 32 (13)	1200 ± 118 (8)	1466 ± 32 (3)
1	N.D.	N.D.	515 ± 35 (3)	N.D.	N.D.
2	N.D.	N.D.	474 ± 22* (8)	736 ± 70** (13)	1152 ± 150 (3)
4	118 ± 13* (12)	240 ± 22 (12)	320 ± 35** (8)	566 ± 48** (12)	976 ± 90** (4)

Table 2. *Estimates of protein synthesis based on the specific radioactivity of aminoacyl-tRNA*

Lungs were perfused for 60 min with buffer equilibrated with $O_2/N_2/CO_2$ (4:15:1) and 4% halothane, as indicated. The specific radioactivity of extracellular and tRNA-bound phenylalanine were determined by using t.l.c. of [^{14}C]dansyl-[^{3}H]phenylalanine, as detailed previously (Watkins & Rannels, 1980). The rate of protein synthesis was calculated as described by McKee *et al.* (1978). Data represent means ± S.E.M. for four observations. $^{**}P < 0.01$ versus control.

Parameter	Condition of perfusion: Control	Condition of perfusion: + Halothane
10^{-2} × [^{3}H]Phenylalanine incorporation (d.p.m./h per mg of protein)	968 ± 46	495 ± 60**
10^{-2} × Phenylalanine specific radioactivity (d.p.m./nmol)		
Extracellular	584 ± 25	530 ± 22
tRNA	520 ± 17	538 ± 51
100 × tRNA/extracellular	90.2 ± 6.5	101.1 ± 7.8
Protein synthesis (nmol of phenylalanine/h per mg of protein) based on the specific radioactivity of:		
Extracellular phenylalanine	1.68 ± 0.13	0.94 ± 0.14**
Phenylalanyl-tRNA	1.85 ± 0.08	0.94 ± 0.19**

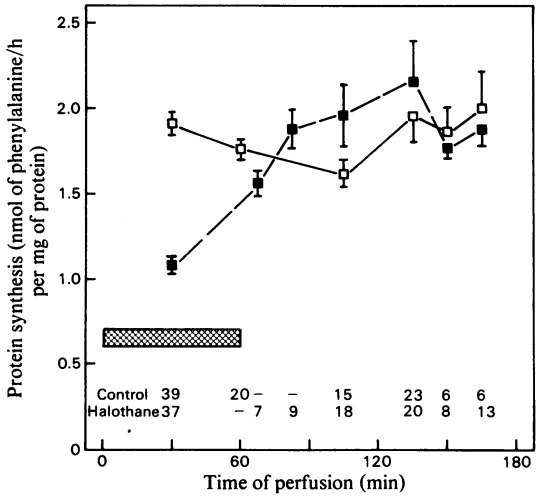

Fig. 1. *Time course of the reversal of the inhibitory effect of halothane on synthesis of lung proteins*

Lungs were perfused as described in the Materials and methods section. Experimental tissues were exposed to $O_2/N_2/CO_2$ (4:15:1) equilibrated with 4% halothane for 60 min, as indicated by the shaded bar. Incorporation of [*ring*-2,6-^{3}H]phenylalanine into protein during the first 60 min *in vitro* was estimated in control (□) and halothane-exposed (■) lungs by removal and analysis of the entire left lung. Tidal volume was then decreased by 40% and delivery of halothane was stopped. Protein synthesis was estimated over subsequent intervals of perfusion by addition of [^{14}C]phenylalanine to the perfusion buffer. Values represent the means ± S.E.M. for the numbers of observations shown at the bottom of the Figure and are plotted at the mid-point of the interval over which protein synthesis was measured. In halothane-exposed lungs, these intervals (min) were: 0–60, 60–75, 75–90, 90–120, 120–150, 120–180 and 150–180.

of anaesthetic, with a 10.7% decrease in the synthesis rate for each 1% of halothane to which the lungs were exposed.

Reversal of the inhibitory effect of halothane

Inhibition of protein synthesis during exposure of the lungs to halothane could have reflected damage to the tissue. Thus the reversibility of the effect was investigated. In these experiments, perfused lungs were exposed to the anaesthetic for 60 min, during which protein synthesis was determined by analysis of [^{3}H]phenylalanine incorporation in the entire left lung, which constitutes 36% of the tissue mass. After delivery of halothane was stopped, perfusion was continued and protein synthesis was again estimated, after allowing various intervals of time for recovery, by adding [^{14}C]phenylalanine to the perfusion medium (Fig. 1). In control lungs, rates of protein synthesis were constant during the 3 h of the experiment. Exposure of the tissues to 4% halothane decreased the synthesis rate by 43%, as observed above. During the first 15 min after halothane delivery was stopped, synthesis increased to 77% of the control rate ($P < 0.001$ versus 4% halothane); it did not differ from the control when measured over the 60–90 min interval, and remained at control values thereafter. These studies showed that the inhibitory effect of halothane on synthesis of lung proteins was rapidly and fully reversible.

Effect of halothane on degradation of lung proteins

In other tissues, rates of protein synthesis and degradation are often regulated in concert (Goldberg & Dice, 1974), although little is known about co-ordinated control of these pathways in lung. Thus the effect of halothane exposure on degradation of lung proteins was investigated by following release

of phenylalanine from lungs perfused with buffer containing cycloheximide. No effect of halothane on phenylalanine release was observed, although the inhibition of protein synthesis (53%) in tissues perfused in parallel studies was somewhat larger than that described above (Table 3). Calculation of the balance of protein turnover, based on the difference between rates of protein synthesis and degradation, suggested that, under the present conditions of perfusion, the lungs were in negative nitrogen balance, with proteolysis exceeding protein synthesis by 30%. This negative balance of protein turnover increased substantially in lungs exposed to halothane, where rates of proteolysis were about 3 times those of protein synthesis (Table 3).

Factors accounting for the inhibitory effect of halothane on synthesis of lung proteins

Measurements of whole-tissue ATP showed that halothane exposure did not result in overall energy depletion in lung (Table 4). Furthermore, concentrations of ATP similar to those in control tissues were observed in another group of lungs 60 min after halothane exposure was stopped, that is, after the effect of the anaesthetic was fully reversed. These observations, taken with the rapid reversibility of the inhibition of protein synthesis and with the lack of an effect of halothane on proteolysis, supported the conclusion that exposure of the lungs to the anaesthetic did not result in extensive tissue damage.

Modification of the properties of lung cell membranes is a likely event during exposure of the tissue to a volatile anaesthetic. Inhibition of amino acid transport or alterations in the availability of amino acids in the intracellular pool resulting from changes in cellular permeability could limit the rate of protein synthesis (Rannels *et al.*, 1974). The extracellular space, as estimated by using [^{3}H]sorbitol, was unchanged after 60 min of exposure to halothane (control, 0.29 ± 0.01 ml/g; 4% halothane, 0.25 ± 0.02 ml/g; C. A. Watkins & D. E. Rannels, unpublished work). Similarly, the half-time of equilibration of [^{14}C]phenylalanine (Watkins *et al.*, 1983), [^{3}H]proline, or the amino acid analogue α-amino-[^{14}C]isobutyric acid (C. A. Watkins & D. E. Rannels, unpublished work) was not altered in halothane-exposed tissues. Data from the proline experiments are shown in Fig. 2. These studies

Table 3. *Effect of halothane on the balance of protein turnover*

Lungs were perfused for 120 min as described in the Materials and methods section and were exposed to 4% halothane, as indicated. For estimates of protein degradation, the perfusate contained 20 μM-cycloheximide and plasma concentrations of amino acids, but no phenylalanine. Protein synthesis was measured as described above; the perfusion buffer contained plasma concentration of 19 amino acids and 690 μM-[^{14}C]phenylalanine. The lungs were assumed to contain 118 mg of protein/g wet wt. (Rannels *et al.*, 1979). The balance of protein turnover was calculated from the difference between rates of protein synthesis and degradation; a negative value represents net release of phenylalanine from the tissue. Data represent means ± S.E.M. for the numbers of observations shown in parentheses. ***$P < 0.001$ versus control.

Parameter	Control	+ Halothane
Protein degradation (nmol of phenylalanine released/g of lung)	578 ± 62 (16)	643 ± 65 (15)
Protein synthesis (nmol of phenylalanine/g of lung)	443 ± 44 (8)	209 ± 18 (12)***
Balance of protein turnover (nmol of phenylalanine/g of lung)	−135	−434

Table 4. *Effect of halothane exposure on lung ATP*

Lungs were perfused for 60 or 120 min and exposed to halothane as indicated. In one group of tissues, halothane exposure was stopped after 60 min and perfusion was continued in the absence of the anaesthetic, as outlined in Fig. 1 ('reversal'). ATP was measured in $HClO_4$ extracts of tissues frozen rapidly, as described in the Materials and methods section. Data represent means ± S.E.M. for the numbers of observations shown in parentheses. Abbreviation: N.D., not determined.

	Lung ATP (μmol/g dry wt.)		
Condition of perfusion	60 min	120 min	120 min, reversal
Control + halothane	7.59 ± 0.43 (10)	6.93 ± 0.65 (12)	6.03 ± 0.48 (4)
1%	7.55 ± 0.32 (3)	N.D.	N.D.
2%	7.46 ± 0.36 (8)	7.00 ± 0.63 (12)	N.D.
4%	6.75 ± 0.61 (8)	6.23 ± 0.42 (12)	7.65 ± 0.76 (5)

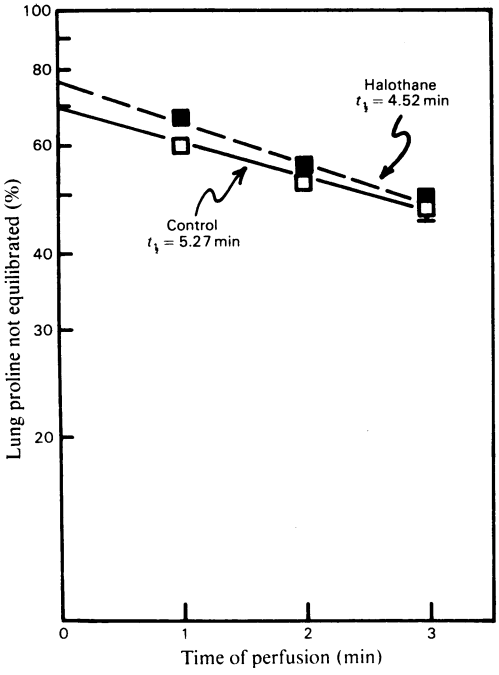

Fig. 2. *Effect of halothane on entry of [^{3}H]proline into perfused lungs*

Lungs were perfused for a preliminary period of 30 min, with (■) or without (□) halothane (4%) exposure. Perfusion was then begun from a separate reservoir with buffer containing [^{3}H]proline (108 μM) and was continued for the period indicated, after which the [^{3}H]proline space was determined. Proline spaces measured at 30 and 60 min were equal and were averaged to represent 100% equilibration. Equilibration of perfusate proline with tissue proline was described by the equations $y = 69e^{-0.132t}$ and $y = 76e^{-0.156t}$ in control and halothane-exposed tissues respectively, where y represents the percentage of lung proline not equilibrated with the perfusate at time t (min). Each point represents the mean ± S.E.M. for 4–11 observations; where S.E.M. is not shown, it did not extend beyond the symbol.

indicated that cellular permeability was not altered drastically in anaesthetic-exposed lungs.

Further experiments suggested that the inhibition of protein synthesis in the presence of halothane was not associated with decreased availability of amino acids in the intracellular pool. When the concentrations of amino acids added to the perfusate were increased to 5 times the plasma value, the extent of the inhibition of protein synthesis by halothane was not diminished (4% halothane inhibited by 41%; $P < 0.01$), although the intracellular concentrations of most amino acids increased 2–5-fold (results not shown). However, not all amino acids increased intracellularly, tryptophan being a notable exception. Even when extracellular tryptophan was 5 times the plasma concentration, intracellular tryptophan did not differ significantly from zero (results not shown), presumably reflecting binding of the amino acid by albumin in the perfusate. Thus the perfusate tryptophan concentration was increased to a value high enough to expand the intracellular tryptophan pool significantly; other amino acids in the perfusate remained at the plasma concentration. Under these conditions, a similar inhibition of protein synthesis by halothane was observed, although intracellular tryptophan was increased to more than 400 μM, in both the presence and the absence of the anaesthetic (Table 5). In the same lungs, intracellular concentrations of seven of the 20 amino acids measured increased after halothane exposure, although no differences in the concentrations of these compounds in the perfusate were noted. The large perfusate-to-intracellular-pool concentration gradients observed for several amino acids (Table 5) further support the conclusion reached above that membrane integrity was preserved in anaesthetic-exposed lungs.

Discussion

The present studies confirm and extend previously reported preliminary observations which suggested that the volatile anaesthetic halothane inhibits protein synthesis in the lung (Wartell *et al.*, 1981). The data in Table 2 provide the first direct confirmation that lower rates of amino acid incorporation into protein in the presence of anaesthetic agents represent an actual decrease in the rate of protein synthesis, rather than a reflection of altered specific radioactivity of the precursor amino acid. This conclusion is based on calculations of the synthesis rate by using the specific radioactivity of aminoacyl-tRNA. Direct considerations of precursor specific radioactivity were not made in any previous studies of the effects of anaesthetics on protein synthesis of which we are aware (Schmidt & Rosenkranz, 1970; Bruce, 1975; Chvapil & Hameroff, 1978; Eichhorn & Peterkofsky, 1979; Hammer & Rannels, 1981; Wartell *et al.*, 1981). The present experiments also confirm the observation that when extracellular concentrations of radioactive phenylalanine are increased to 690 μM, ten times the plasma value, the contribution of proteolysis-derived amino acids to protein synthesis is negligible (Watkins & Rannels, 1980). A similar equilibration between tRNA-bound and extracellular phenylalanine specific radioactivities was reached in the presence of halothane.

The inhibitory effect of halothane on the synthesis of lung proteins was reversed rapidly, probably within the first 15 min after delivery of the anaesthetic was stopped. Rapid reversal of the

Table 5. *Effect of halothane on intracellular amino acid concentrations in lung*

Lungs were perfused for 60 min as described in the Materials and methods section, except that 2 mM-tryptophan was added to the perfusate. Other amino acids were present at the plasma concentrations. Halothane (4%) was present as indicated. Data represent means ± S.E.M. for the numbers of observations shown in parentheses. $*P < 0.05$ and $**P < 0.01$ versus control.

	Amino acid concentration (μM)			
	Extracellular		Intracellular	
Amino acid measured	Control (5)	+ Halothane (6)	Control (5)	+ Halothane (6)
Aspartic acid	26.6 ± 1.4	23.7 ± 1.1	3529 ± 260	4636 ± 345*
Threonine	190.7 ± 1.5	186.8 ± 2.4	1327 ± 101	1522 ± 73
Serine	210.3 ± 4.2	202.2 ± 8.2	1699 ± 128	1747 ± 46
Asparagine	58.9 ± 3.1	58.0 ± 3.9	430 ± 29	515 ± 31
Glutamine	513.7 ± 6.2	511.9 ± 7.8	4333 ± 350	4628 ± 217
Proline	91.4 ± 2.4	90.2 ± 1.6	268 ± 24	454 ± 42**
Glutamic acid	150.1 ± 3.9	143.0 ± 2.4	8320 ± 643	6950 ± 348
Glycine	308.6 ± 2.2	304.3 ± 2.2	12313 ± 836	14079 ± 944
Alanine	317.7 ± 0.7	318.6 ± 2.5	2489 ± 229	3047 ± 193
Valine	179.2 ± 0.4	178.3 ± 1.9	836 ± 146	1081 ± 163
Half-cystine	37.6 ± 1.0	34.4 ± 1.6	17 ± 5	25 ± 1
Methionine	46.8 ± 1.2	45.3 ± 1.4	84 ± 3	116 ± 10*
Isoleucine	95.9 ± 0.9	95.6 ± 1.2	144 ± 11	166 ± 12
Leucine	141.7 ± 1.0	140.3 ± 1.5	251 ± 10	319 ± 20*
Tyrosine	42.5 ± 0.2	42.1 ± 0.3	106 ± 2	155 ± 12**
Phenylalanine	609.5 ± 3.2	607.2 ± 4.4	737 ± 32	754 ± 24
Lysine	336.9 ± 5.0	337.4 ± 3.7	290 ± 49	378 ± 25
Histidine	46.4 ± 0.6	46.4 ± 0.4	96 ± 4	120 ± 5**
Tryptophan	637.1 ± 4.4	630.4 ± 5.1	410 ± 53	414 ± 30
Arginine	97.0 ± 1.2	97.0 ± 1.9	122 ± 5	172 ± 11**

halothane-mediated inhibition of phenylalanine incorporation was observed previously in primary cultures of mixed lung cells (Wartell *et al.*, 1981) and in pulmonary macrophages (Hammer & Rannels, 1981). Rates of synthesis in lungs previously exposed to halothane did not differ significantly from those in control tissues, but tended to be higher than control values (Fig. 1). In some experiments, rates of synthesis measured 30–60 min after a 1 h exposure of the lungs to halothane were significantly higher than those in control tissues ($P < 0.01$); this overshoot in the synthesis rate was not observed consistently, nor is information available about its potential mechanism.

Previously published studies in cultured mixed lung cells (Wartell *et al.*, 1981) and pulmonary macrophages (Hammer & Rannels, 1981) indicated that the effects of halothane are exerted at the cellular level and do not reflect alterations in perfusion of the pulmonary vascular bed resulting from anaesthetic-mediated changes in vascular resistance. Measurements of pulmonary flow at a constant perfusion pressure of 20 cm H_2O indicated that overall vascular resistance is not altered substantially in the present preparation by halothane (Wartell *et al.*, 1981). Evidence obtained in both intact lung tissue and isolated lung cells indicated that exposure to the anaesthetic does not lead to extensive or irreversible damage, further suggesting that the effects of the drug are exerted in some specific fashion at the cellular level.

Halothane exposure did not alter the rate of degradation of lung proteins. Although the overall regulation of proteolysis in lung tissue has not been much investigated, hypoxia inhibits protein degradation in the lung (Chiang *et al.*, 1981) and other tissues (Chua *et al.*, 1979). In the latter case, these effects are associated with accumulation of lactate and H^+ ions, products of anaerobic metabolism. Together, these observations further suggest that halothane exposure does not lead to regional hypoxia in lung tissue resulting from altered perfusion. Other studies showed that halothane did not inhibit the degradation of endogenous proteins or of exogenous bovine serum albumin by pulmonary macrophages (Hammer & Rannels, 1981). Local anaesthetics, on the other hand, may exert inhibitory effects on the proteolytic pathway (Carpenter & Cohen, 1976; Dean, 1979).

A variety of mechanisms could be proposed to

account for the effect of halothane on synthesis of lung proteins. Anaesthetic agents are well known to affect cellular metabolism through several pathways (Cohen, 1973; Biebuyck & Lund, 1974; Hallén & Johansson, 1975), as well as to exert effects on cellular membranes (Trudell & Cohen, 1975) and protein conformation (Eyring *et al.*, 1973). Difficulties in selecting among these possibilities as a basis for the inhibitory effect of halothane result primarily from the limited information available about the control of protein synthesis in lung. The present results suggest that changes in the availability of amino acids as substrates for the pathway of protein synthesis are not likely to account for the effect of the anaesthetic. Both control and halothane-exposed lungs were supplied with a large pool of amino acids extracellularly and the intracellular concentration of amino acids was not decreased when halothane was present. Furthermore, the inhibitory effect of the anaesthetic was unchanged when the extracellular concentrations of amino acids were raised 5 times above physiological values.

The possibility that tryptophan, which is present in plasma at low concentrations, limited the synthesis pathway was further investigated because this amino acid appears to become rate-limiting for synthesis of liver proteins in starved animals and during amino acid deprivation *in vitro* (Wunner *et al.*, 1966; Jefferson & Korner, 1969). The role of tryptophan availability in limiting synthesis of lung proteins is controversial (Gacad *et al.*, 1972; Rannels *et al.*, 1979). In addition, halothane inhibits the uptake of 5-hydroxytryptamine by the intact lung by increasing the apparent K_m of the transport system for this amine (Watkins *et al.*, 1983), which is structurally similar to tryptophan. However, the inhibitory effect of halothane on protein synthesis was observed under conditions where the extracellular tryptophan concentration was raised sufficiently to enlarge the size of the intracellular pool of the amino acid well beyond that present in control lungs. These observations are consistent with other experiments which showed that, in lungs perfused with buffer containing bovine serum albumin (fraction V), increasing the concentration of 20 amino acids in the perfusion buffer to 5 times the plasma value did not affect synthesis of lung proteins (J. M. Besterman, C. A. Watkins & D. E. Rannels, unpublished work).

The possibility that alterations in calcium metabolism were involved in the mechanism of the inhibitory effects of halothane was considered, because general anaesthetics decrease the sensitivity of potassium-depolarized blood vessels to $CaCl_2$-induced contraction and inhibit the Ca^{2+}-dependent spontaneous mechanical activity involved in maintenance of vascular smooth-muscle tone (Altura & Altura, 1981). This link between anaesthetic action and Ca^{2+} metabolism is further supported by more direct studies (Nayler & Szeto, 1972; Turlapaty *et al.*, 1979; Altura *et al.*, 1980), including the observation that Ca^{2+} reverses the halothane-induced depression of papillary-muscle contractility (Price, 1974). These relationships are of interest in light of the observation that the Ca^{2+} antagonist verapamil (10–100μM) decreased the incorporation of radioactive proline into collagenase-digestible and non-collagen proteins by foetal-rat calvaria (Dietrich & Duffield, 1979). Synthesis of skeletal-muscle proteins increased in the presence of the Ca^{2+} ionophore A23187 (Kameyama & Etlinger, 1979; Roufa *et al.*, 1981). Synthesis in incubated muscles was unaffected by 2mM-EGTA (without added Ca^{2+}), and was not increased by the ionophore in the presence of the chelator (Kameyama & Etlinger, 1979).

In perfused lungs, increasing extracellular Ca^{2+} 4-fold had no effect on protein synthesis [2.5mM-Ca^{2+}, 1.76 ± 0.11 (S.E.M.) nmol of phenylalanine incorporated/h per mg of protein, $n = 17$; 10.5mM-Ca^{2+}, 1.85 ± 0.13, $n = 9$]. Similarly, synthesis was unchanged in tissues supplied perfusate without added Ca^{2+}, but containing 0.5mM-EDTA (1.62 ± 0.13, $n = 4$), or with perfusate containing verapamil (100μM; 1.68 ± 0.12, $n = 9$). Diltiazem (100μM), a second Ca^{2+} antagonist, decreased synthesis by 30% (1.26 ± 0.08, $n = 9$; $P < 0.005$ versus control). Attempts to reverse or prevent the halothane-mediated inhibition of protein synthesis with the Ca^{2+} ionophore A23187 (10μg/ml) were unsuccessful; lungs exposed to the drug rapidly developed severe oedema, which blocked pulmonary flow. Although the results with diltiazem are consistent with the hypothesis that halothane may act through modulation of cellular Ca^{2+}, no supporting evidence is available. Clearly, the drawing of any conclusions about the role of the cation in the inhibitory effect of halothane requires more detailed studies of the role of this and other factors in the regulation of protein synthesis in lung.

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References

Altura, B. M. & Altura, B. T. (1981) in *New Perspectives on Calcium Antagonists* (Weiss, E. B., ed.), pp. 131–145, American Physiological Society, Bethesda

Altura, B. T., Turlapaty, P. D. M. V. & Altura, B. M. (1980) *Biochim. Biophys. Acta* **595**, 309–312
Bakhle, Y. S. & Block, A. J. (1976) *Clin. Sci. Mol. Med.* **50**, 87–90
Biebuyck, J. F. & Lund, P. (1974) *Mol. Pharmacol.* **10**, 474–483
Biebuyck, J. F., Lund, P. & Krebs, H. A. (1972*a*) *Biochem. J.* **128**, 711–720
Biebuyck, J. F., Lund, P. & Krebs, H. A. (1972*b*) *Biochem. J.* **128**, 721–723
Bruce, D. L. (1975) *Anesthesiology* **42**, 11–14
Carpenter, G. & Cohen, S. (1976) *J. Cell Biol.* **71**, 159–171
Chiang, M. J., Kishi, F., Whitney, P. & Massaro, D. (1981) *Am. J. Physiol.* **241**, E101–E107
Chua, B., Kao, R. L., Rannels, D. E. & Morgan, H. E. (1979) *J. Biol. Chem.* **254**, 6617–6623
Chvapil, M. & Hameroff, S. R. (1978) *Am. Soc. Anesth. Abstr.* 139–140
Cohen, E. N. (1977) *ASA Refresher Courses in Anesthesiology* **5**, 21–32
Cohen, P. J. (1973) *Anesthesiology* **39**, 153–164
Dean, R. T. (1979) *Biochem. J.* **180**, 339–345
Dietrich, J. W. & Duffield, R. (1979) *Endocrinology* **105**, 1168–1172
Eichhorn, J. H. & Peterkofsky, B. (1979) *J. Cell Biol.* **81**, 26–42
Eyring, H., Woodbury, J. W. & D'Arrigo, J. S. (1973) *Anesthesiology* **38**, 415–424
Gacad, G., Dickie, K. & Massaro, D. (1972) *J. Appl. Physiol.* **33**, 381–384
Goldberg, A. L. & Dice, J. F. (1974) *Annu. Rev. Biochem.* **43**, 835–869
Hallén, B. & Johansson, G. (1975) *Anesthesiology* **43**, 34–40
Hammer, J. A. & Rannels, D. E. (1981) *Am. Rev. Respir. Dis.* **124**, 50–55
Jefferson, L. S. & Korner, A. (1969) *Biochem. J.* **111**, 703–712
Kameyama, T. & Etlinger, J. D. (1979) *Nature (London)* **279**, 344–346
McKee, E. E., Cheung, J. Y., Rannels, D. E. & Morgan, H. E. (1978) *J. Biol. Chem.* **253**, 1030–1040
McLain, G. E., Sipes, I. G. & Brown, B. R., Jr. (1979) *Anesthesiology* **51**, 321–326
Nahrwold, M. L. & Cohen, P. J. (1973) *Anesthesiology* **39**, 534–536
Naito, H. & Gillis, C. N. (1973) *Anesthesiology* **39**, 575–580
Nayler, W. G. & Szeto, J. (1972) *Am. J. Physiol.* **222**, 339–344
Price, H. L. (1974) *Anesthesiology* **41**, 576–579
Rannels, D. E., Li, J. B., Morgan, H. E. & Jefferson, L. S. (1974) *Methods Enzymol.* **37B**, 238–250
Rannels, D. E., Kao, R. & Morgan, H. E. (1975) *J. Biol. Chem.* **250**, 1694–1701
Rannels, D. E., McKee, E. E. & Morgan, H. E. (1977) *Biochem. Actions Horm.* **4**, 135–195
Rannels, D. E., Sahms, R. H. & Watkins, C. A. (1979) *Am. J. Physiol.* **236**, E421–E428
Rannels, D. E., Roake, G. M. & Watkins, C. A. (1982*a*) *Anesthesiology* **57**, 87–93
Rannels, D. E., Wartell, S. A. & Watkins, C. A. (1982*b*) *Life Sci.* **30**, 1679–1690
Ross, W. T., Daggy, B. P. & Cardell, R. R., Jr. (1979) *Anesthesiology* **51**, 327–333
Roufa, D., Wu, F. S. & Martonosi, A. M. (1981) *Biochim. Biophys. Acta* **674**, 225–237
Schmidt, R. M. & Rosenkranz, H. S. (1970) *J. Infect. Dis.* **121**, 597–607
Trudell, J. R. & Cohen, E. N. (1975) *Prog. Anesthesiol.* **1**, 315–321
Turlapaty, P. D. M. V., Altura, B. T. & Altura, B. M. (1979) *Experientia* **35**, 639–640
Wartell, S. A., Christopherson, R., Watkins, C. A. & Rannels, D. E. (1981) *Mol. Pharmacol.* **19**, 520–524
Watkins, C. A. & Rannels, D. E. (1979) *J. Appl. Physiol.* **47**, 325–329
Watkins, C. A. & Rannels, D. E. (1980) *Biochem. J.* **188**, 269–278
Watkins, C. A., Wartell, S. A. & Rannels, D. E. (1983) *Biochem. J.* **210**, 157–166
Wozniak, M. (1978) *Biochem. Pharmacol.* **27**, 2959–2961
Wunner, W. H., Bell, J. & Munro, H. N. (1966) *Biochem. J.* **101**, 417–428