MOLECULAR PHARMACOLOGY, 19:520–524

Inhibition of Synthesis of Lung Proteins by Halothane

SUE A. WARTELL,[1] ROSE CHRISTOPHERSON,[2] CLYDE A. WATKINS, AND D. EUGENE RANNELS[3]

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

Received November 12, 1980; Accepted January 21, 1981

SUMMARY

WARTELL, S. A., R. CHRISTOPHERSON, C. A. WATKINS, AND D. E. RANNELS. Inhibition of synthesis of lung proteins by halothane. Mol. Pharmacol. **19**:520–524 (1981).

The effect of halothane exposure on synthesis of lung proteins was investigated. In rat lungs perfused *in situ* with Krebs-Henseleit bicarbonate buffer containing plasma levels of 19 amino acids, 690 μM [^{14}C]phenylalanine, 5.6 mM glucose, and 4.5% bovine serum albumin, protein synthesis was linear for at least 4 hr. Halothane (1–4% equilibrated with $O_2/N_2/CO_2$, 4:15:1) rapidly inhibited protein synthesis in a dose-dependent manner, with about a 10% depression of the rate for each 1% increment in halothane. The inhibition was rapidly and completely reversed when halothane delivery was stopped; it was not associated with depletion of tissue ATP or with nonspecific changes in cellular permeability. A similar reversible and dose-dependent inhibition of protein synthesis was observed in primary cultures of mixed lung cells incubated in Dulbecco's minimal essential medium containing 10% donor calf serum. These results suggested a significant but reversible inhibition of protein synthesis, exerted at the cellular level, in lungs exposed to halothane.

Halothane, a frequently used volatile anesthetic, is known to affect liver metabolism at the microsomal (1), mitochondrial (2), and glycogenolytic (3) levels. Although the lungs are the primary site of exposure to halothane during surgical anesthesia, little information is available regarding the effects of volatile anesthetics on lung metabolism. Similarly, the effects of anesthetics on protein metabolism have received little attention. Schmidt and Rosenkranz (4) linked the antimicrobial activities of lidocaine and procaine to inhibition of protein, RNA, and DNA synthesis. Preliminary studies on skin slices from newborn rats indicated that lidocaine inhibited incorporation of [^{14}C]proline into collagen and noncollagen protein (5). Finally, Bruce (6) reported that overnight exposure of cultured human lymphocytes to 2% halothane inhibited the PHA[4]-induced increase in [^{14}C]leucine incorporation. The effect of halothane exposure was observed 16 hr following PHA treatment; halothane effects in nontreated cells (without PHA) were not reported.

In the present studies, the effect of halothane exposure on protein synthesis in lung was investigated. Rates of protein synthesis were estimated in perfused lungs and in cultured lung cells under conditions which minimized uncertainty as to the specific radioactivity of precursor amino acids. These studies showed that exposure of intact lung tissue or isolated lung cells to halothane led to a rapid and dose-dependent inhibition of protein synthesis which did not result from nonspecific cell damage.

Male Sprague-Dawley rats (175–200 g) obtained from Charles River Breeding Laboratories, Wilmington, Mass., were provided Agway RMH 3000 chow and water ad libitum. Following i.p. injection of sodium pentobarbital (50 mg/kg body weight), the animals were weighed and surgically prepared for lung perfusion as detailed earlier (7). The first 30 ml of perfusate to pass through the lungs were discarded; as recirculation of the perfusate (100 ml) was begun, radioactive phenylalanine was added to the buffer reservoir. Previous studies have shown that this preparation is stable for at least 240 min of perfusion (7).

The perfusate was a modified Krebs-Henseleit bicarbonate buffer containing 4.5% (w:v) bovine serum albumin (Pentex, Fraction V; Miles Laboratories, Elkhart, Ind.), 5.6 mM glucose, and 19 amino acids at levels measured in rat plasma (8, 9). Perfusate phenylalanine was 690 μM, 10 times the plasma level (specific radioac-

This work was supported by Grant HL-20344 and by Pulmonary Specialized Center of Research Grant HL-19190 from the National Institutes of Health.

[1] Postdoctoral Research Fellow of the Parker B. Francis Foundation, Hanover, N. H.

[2] Supported by a stipend from the Research Division of the Department of Anesthesia.

[3] Recipient of Research Career Development Award HL-00294.

[4] The abbreviations used are: MEM, minimal essential medium; MEM-D, Dulbecco's modified minimal essential medium; PHA, phytohemagglutinin; TCA, trichloroacetic acid

0026-895X/81/030520-05$02.00/0

tivity, 320 dpm/nmole). Previous studies (9) have shown that, at this concentration of extracellular phenylalanine, the specific radioactivity of phenylalanyl-tRNA is equal to that of phenylalanine in the perfusate. Thus, rates of protein synthesis could be calculated accurately on the basis of the specific radioactivity of extracellular phenylalanine. The perfusate was equilibrated with warmed (37°), humidified $O_2/N_2/CO_2$ (4:15:1); halothane (2-bromo-2-chloro-1,1,1-trifluoroethane; Fluothane, Ayerst Laboratories, Inc., New York, N. Y.) was mixed with this gas mixture as indicated, using a Fluotec 3 vaporizer (Fraser Sweatman, Inc., Lancaster, N. Y.). The concentration of anesthetic in the perfusate and in cell media (below) was measured using a Hewlett-Packard 5840-A gas chromatograph standarized against halothane solutions of known concentration (10). Incorporation of radioactive phenylalanine into lung protein, tissue levels of ATP, and amino acid spaces were determined as described earlier (7, 9).

The preparation of mixed lung cells was based on modifications of the approaches of Kikkawa and Yoneda (11) and of Douglas and Teel (12). Following thoracotomy, inflated rat lungs were perfused *in situ* with 0.15 M NaCl solution (25°). The lungs were removed, minced thoroughly, and washed with 100 ml of Joklik's MEM (Grand Island Biological Company, Grand Island, N. Y.). The suspended mince was filtered on HC-160 nylon bolting cloth and combined with digestion medium containing (final concentration) collagenase (0.1%; Worthington Biochemical Corporation, Freehold, N. J.), bacterial neutral protease (dispase, 0.05%; Boehringer Mannheim Biochemicals, Indianapolis, Ind.), DNAse (0.0067%; Sigma, $BaSO_4$ (0.033%), and chick serum (1.0%; Grand Island Biological Company or Flow Laboratories, Rockville, Md.). Digestion at 37° resulted in a high yield of cells ($\sim 2 \times 10^8$/rat), of which >95% were viable, as judged by exclusion of erythrocin B (13). Cells were plated ($\sim 10^7$/60-mm plate) in MEM-D containing 10% (v:v) donor bovine serum (Flow Laboratories). After overnight incubation (37°) in humidified CO_2/air (1:9), nonadhered cells were removed and fresh experimental medium was added. About 40% of the protein and DNA applied to the plates remained attached. The adhered cell population contained about 13% macrophages, 15% fibroblastic cells, 8% Type II granular pneumocytes, 33% other epithelial cells, and 31% cell types unidentified in hematoxylin/$LiCO_3$-stained preparations.

For determinations of [^{14}C]amino acid incorporation into protein, the incubation medium was removed and cellular protein was precipitated with cold 10% TCA. Hot TCA-insoluble material was collected on Millipore filters (HAWP; pore size 0.45 μm), and washed with 5% TCA. The filters were incubated in 0.8 ml of 88% formic acid at room temperature (4 hr) and radioactivity was determined in 10 ml of Formula 947 (New England Nuclear Corporation, Boston, Mass.). The value for disentegrations per minute incorporated per milligram of protein was calculated on the basis of efficiency corrections using an external standard and on protein determinations run on separate plates (0.178 ± 0.019 mg of protein/plate), as described by Lowry *et al.* (14).

Freshly isolated mixed lung cells were prepared and placed in culture as described above. After overnight incubation and removal of nonadhered cells, 5.0 ml of fresh MEM-D containing 10% donor calf serum was added and the plates were transferred to temperature-controlled chambers (37°). Halothane was delivered with the use of a Fluotec 3 vaporizer in a gas mixture containing $O_2/N_2/CO_2$ (4:15:1). Equilibration of the medium with the vapor phase was complete within 10 min.

Data were compared using Student's *t*-test. A p value <0.05 was considered significant.

Protein synthesis in rat lungs perfused *in situ* under control conditions was linear during at least 120 min *in vitro* (Fig. 1). Phenylalanine was incorporated into lung protein at a rate of 1.74 ± 0.10 nmoles/mg of protein per hour, in good agreement with earlier studies (9, 15, 16). Exposure of the lungs to 4% halothane did not modify the linearity of the time course, but inhibited protein synthesis by 43%, to 0.99 ± 0.09 nmole of phenylalanine incorporated/mg of protein per hour. With 2% halothane, intermediate rates of synthesis were observed (Fig. 1). Extrapolation of the time course of protein synthesis to the initiation of halothane exposure suggested that the effect of the anesthetic was rapid in onset, since no lag in the development of the inhibition was evident. The magnitude of the effect of halothane was linearly related to the amount of the anesthetic presented (Fig. 2). For each

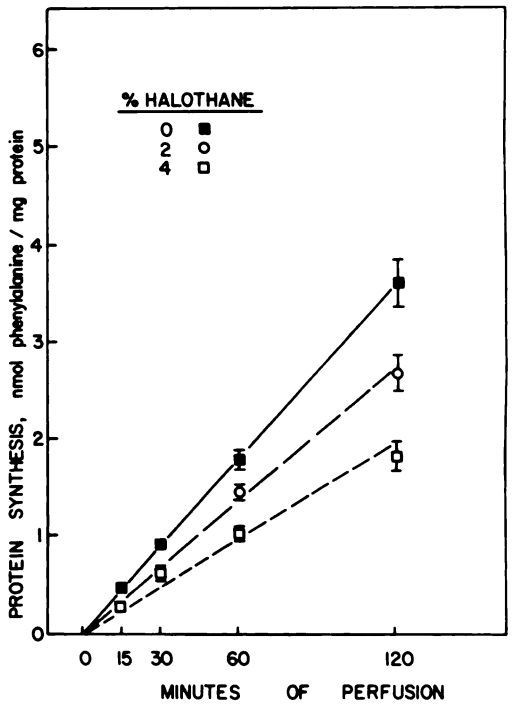

FIG. 1. *Effect of halothane on protein synthesis in perfused lungs*
Lungs were perfused 15 to 120 min as described in text. In control experiments, the perfusate was equilibrated with $O_2/N_2/CO_2$ (4:15:1); halothane was added to this gas mixture as indicated. Under these conditions, halothane concentrations measured in the perfusate leaving the pulmonary circulation were 1.44 ± 0.15, 0.88 ± 0.12, and 0.46 ± 0.07 mM (three observations each) at vaporizer settings of 4, 2, and 1%, respectively. At the 4% setting, direct sampling of the perfusate reservoir showed a halothane concentration of 2.77 ± 0.03 mM (seven observations). Each point represents the mean ± standard error of the mean of six to eight observations.

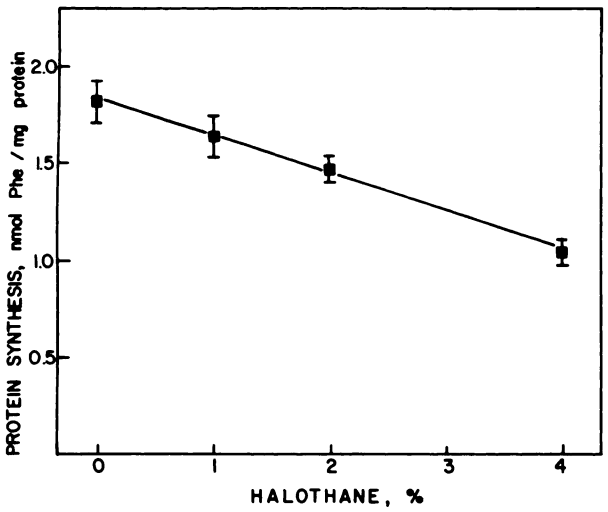

FIG. 2. *Dose-response relationship of the inhibition of protein synthesis by halothane in perfused lungs*

Lungs were perfused for 60 min and halothane was presented as described in text. The *line* in the figure was drawn by least-squares linear regression. Each point represents the mean ± standard error of the mean of six to eight observations.

1% increment in halothane concentration, protein synthesis was inhibited about 10%.

Halothane exposure did not result in over-all energy depletion in the preparation. No significant difference was observed in the ATP content of lungs perfused for 120 min in the presence or absence of halothane [control, 6.9 ± 0.7 μmoles/g of dry weight (n = 12); plus halothane, 6.2 ± 0.4 μmoles/g of dry weight (n = 12)]. Exposure of the lung to the anesthetic did not lead to general changes in cellular permeability. When transport of extracellular phenylalanine or proline into the tissues was examined, neither the half-time of equilibration nor the equilibrium spaces of these amino acids were affected by halothane. After 60 min of perfusion, [^{14}C]phenylalanine spaces were 0.71 ± 0.05 (n = 6) and 0.74 ± 0.03 (n = 5) ml/g in control and halothane-exposed lungs, respectively. In similar experiments, [^{3}H]proline spaces were 0.90 ± 0.06 (n = 6) and 0.90 ± 0.04 (n = 5) ml/g. Taken together, these results indicated that exposure of the lungs to halothane did not result in extensive cell damage.

Furthermore, the inhibition of protein synthesis by halothane was rapidly reversed on removal of the anesthetic. Synthetic rates during and after halothane exposure were measured directly in the same lungs using a double-isotope approach (Fig. 3). Incorporation of [^{3}H]phenylalanine was measured after 60 min of perfusion (± halothane) by tying off and removing the single lobe of the left lung for analysis. Control experiments showed that this procedure did not alter the rate of protein synthesis in the remaining lobes of the right lung. In these tissues, the inhibitory effect of halothane was similar to that discussed above (Fig. 3, *left bars*). After delivery of the anesthetic was stopped (at 60 min), recirculation of buffer containing [^{3}H]phenylalanine was continued and, at 90 min, [^{14}C]phenylalanine was added to the perfusate. Measurements of [^{14}C]phenylalanine incorporation over the final 30 min of perfusion (90–120 min) revealed similar rates of protein synthesis in control tissues and in lungs previously exposed to halothane (Fig. 3, *right bars*). Calculations of the synthetic rate during the interval from 60 to 90 min of perfusion (based on total ^{3}H incorporation), suggested that reversal of the halothane effect was rapid.

Halothane is known to alter vascular resistance in some tissues (17). Over-all vascular resistance in the perfused lungs was not altered by the anesthetic, since at 20 cm H_2O pulmonary arterial pressure, the rate of perfusate flow was unchanged by halothane (control, 36 ± 2; halothane-exposed, 38 ± 1 ml/min). However, a redistribution of perfusate flow could not be ruled out by these observations. The effect of halothane exposure on a primary culture of mixed lung cells was therefore investigated. This preparation was chosen to approximate the cellular composition of the intact lung and to preclude selection of an insensitive cell type. Initial experiments, similar to those detailed for perfused lungs (9), suggested that, when extracellular [^{14}C]phenylalanine was at or above 400 μM, dilution of the radioactive precursor with phenylalanine derived from proteolysis was minimal. Thus, subsequent studies were performed using 400 μM phenylalanine, the concentration found in commercially prepared Dulbecco's MEM; apparent rates of protein synthesis were calculated on the basis of the specific radioactivity of phenylalanine in the medium (9).

Exposure of cell cultures to 4% halothane inhibited protein synthesis to 65 ± 3% of the control rate (p <

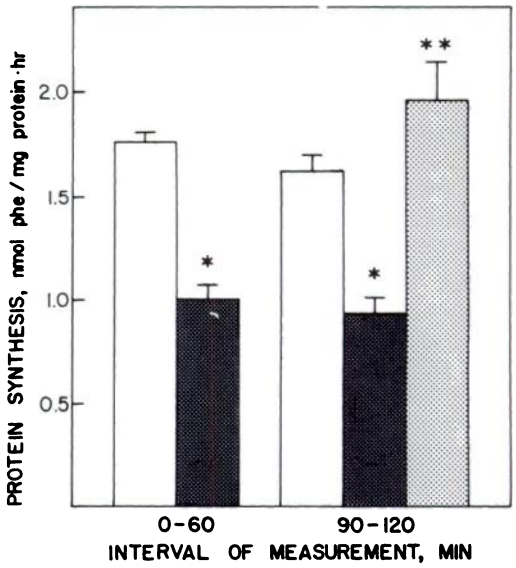

FIG. 3. *Reversibility of the effect of halothane on the synthesis of lung proteins*

Lungs were perfused as described in legend to Fig. 1. Incorporation of [ring 2,6-^{3}H(N)]phenylalanine into protein during the first 60 min *in vitro* was estimated in control (*open bars*) and 4% halothane-exposed (*dark bars*) lungs by removal and analysis of the entire left lung. Tidal volume was decreased by 40% and, in some lungs, the delivery of halothane was stopped. At 90 min, [U-^{14}C]phenylalanine was added to the perfusate to determine the rate of protein synthesis over the final 30-min interval of perfusion. Data from lungs which were exposed to halothane during only the first 60 min are shown by the *shaded bar*. Values represent the mean ± standard error of the mean of 7–21 observations. Similar results were obtained when the order of addition of the isotopes was reversed. * $p < 0.001$ versus control; ** $p < 0.001$ versus halothane.

0.001). The extent of inhibition was dose-dependent (Fig. 4) and of a magnitude similar to that described above. Thus, the halothane effects observed in perfused tissues appeared to be exerted directly at the cellular level and were not secondary to alterations in perfusion parameters. Additional experiments showed that, as observed in perfused lungs, the inhibition was fully reversible when halothane delivery to the cell cultures was stopped; the rate from 40 to 160 min after removal of halothane was 103 ± 12% of control (17 observations).

Although halothane is a commonly used volatile anesthetic, its effects on metabolic function of the lung have received little attention. The present experiments demonstrate that, at clinical doses, halothane exposure inhibits the incorporation of [^{14}C]phenylalanine into lung proteins. Rates of protein synthesis were estimated in the presence of high extracellular levels of the precursor amino acid, [^{14}C]phenylalanine. In perfused lungs (9), as in other tissues (for review, see ref. 18), provision of high levels of precursor minimized the dilution of the specific radioactivity of intracellular phenylalanine and aminoacyl-tRNA by amino acids derived from protein breakdown and allowed rates of protein synthesis to be calculated accurately according to the specific radioactivity of extracellular amino acid. This approach was applied to lung cells in the present experiments, although specific radioactivities of extracellular and tRNA-bound phenylalanine were not compared directly.

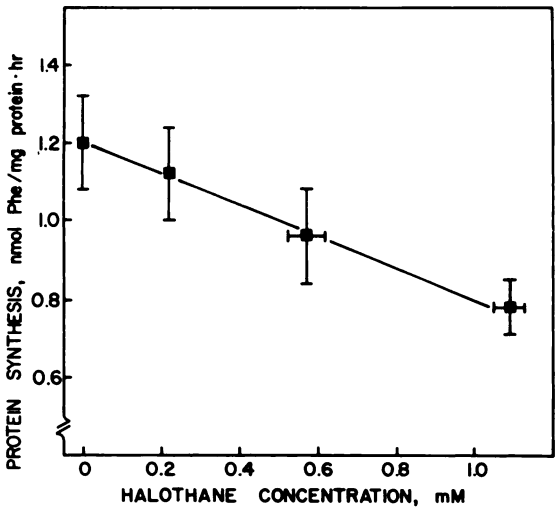

FIG. 4. *Effect of halothane on protein synthesis in cultured lung cells*

Primary cultures of mixed lung cells were incubated ± halothane as outlined in text. After 15 min, [^{14}C]phenylalanine was added to the incubation medium and protein synthesis was estimated over the next 30–60 min. Halothane concentration was measured in medium removed from each plate at the end of the incorporation period. Halothane exposure (+H) did not alter the number of cells per plate (control, 9.06 ± 0.18 × 10^5; +H, 8.81 ± 0.14 × 10^5; eight observations each) or cell viability, as judged from exclusion of erythrocin-B (control, 100%; +H, 99.3% viable). The *line* was determined by least-squares linear regression; the equation is $y = -0.39\,x + 1.20$ ($r^2 = -0.998$), where y is protein synthesis (nanomoles of phenylalanine per milligram of protein per hour) and x is halothane concentration (millimolar). Values are from one cell preparation and represent the mean ± standard error of the mean of eight observations. While the apparent rate of protein synthesis varied among cell preparations, the percentage inhibition of protein synthesis by halothane was independent of the control rate of synthesis (three experiments). Where the standard error of the mean is not shown, it did not extend beyond the symbol.

The present preliminary experiments provided some indirect evidence as to the mechanism by which halothane reduces protein synthesis. Inhibition of protein synthesis in primary cultures of mixed lung cells eliminated redistribution of pulmonary circulation as a cause of the inhibition. The rapid changes in protein synthesis observed suggested that the efficiency of messenger RNA translation was altered, in contrast with the previously reported longer-term effects of starvation or unilateral pneumonectomy, which involved changes in tissue levels of RNA (15, 16). Rapid and complete reversibility of the halothane effect as well as direct measurements of cell viability suggested that the inhibition did not involve cell death. Although tissue levels of ATP were unchanged by halothane administration in perfused lungs, local alterations in high-energy phosphates could not be eliminated on the basis of these observations. More extensive measurements of the effects of the anesthetic on energy levels in specific isolated lung cell types is required before localized energy depletion can be fully eliminated as a cause of the inhibition.

Anesthetic agents may act upon the cell, either directly or indirectly, through alterations of energy metabolism (2, 19), by affecting physiochemical changes in membrane volume (20), fluidity (21), or protein content (22), or by direct effects on protein conformation (23). Halothane is known to modify specific membrane-linked events associated with the vascular endothelium in intact lungs (24–26); potential links between the membrane effects of the anesthetic and the mechanism of the present observations remain to be investigated.

ACKNOWLEDGMENT

The authors thank Ms. Patricia A. Gering for typing the manuscript.

REFERENCES

1. Hallén, B., and G. Johansson. Inhalation anesthetics and cytochrome P-450-dependent reactions in rat liver microsomes. *Anesthesiology* **43**:34–40 (1975).
2. Cohen, P. J. Effect of anesthetics on mitochondrial function. *Anesthesiology* **39**:153–164 (1973).
3. Biebuyck, J. F., and P. Lund. Effects of halothane and other anesthetic agents on the concentrations of rat liver metabolites *in vivo*. *Mol. Pharmacol.* **10**: 474–483 (1974).
4. Schmidt, R. M., and H. S. Rosenkranz. Antimicrobial activity of local anesthetics: lidocaine and procaine. *J. Infect. Dis.* **121**:597–607 (1970).
5. Chvapil, M., and S. R. Hameroff. Lidocaine effects on collagen synthesis. *Am. Soc. Anesth. Abstr.* 139–140 (1978).
6. Bruce, D. L. Halothane inhibition of RNA and protein synthesis of PHA-treated human lymphocytes. *Anesthesiology* **42**:11–14 (1975).
7. Watkins, C. A., and D. E. Rannels. *In situ* perfusion of rat lungs: stability and effects of oxygen tension. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* **47**:325–329 (1979).
8. Morgan, H. E., L. S. Jefferson, E. B. Wolpert, and D. E. Rannels. Regulation of protein synthesis in heart muscle: II. Effect of amino acid levels and insulin on ribosomal aggregation. *J. Biol. Chem.* **246**:2163–2170 (1971).
9. Watkins, C. A., and D. E. Rannels. Measurement of protein synthesis in rat lungs perfused *in situ*. *Biochem. J.* **188**:269–278 (1980).
10. Hammer, J. A., III, and D. E. Rannels. Effects of halothane on protein synthesis and degradation in rabbit pulmonary macrophages. *Am. Rev. Respir. Dis.*, in press (1981).
11. Kikkawa, Y., and K. Yoneda. The type II epithelial cell of the lung. I. Method of isolation. *Lab. Invest.* **30**:76–84 (1974).
12. Douglas, W. H. J., and R. W. Teel. An organotypic *in vitro* model system for studying pulmonary surfactant production by type II alveolar pneumocytes. *Am. Rev. Respir. Dis.* **113**:17–23 (1976).
13. Phillips, H. J., and J. E. Terryberry. Counting actively metabolizing tissue cultured cells. *Exp. Cell Res.* **13**:341–347 (1957).
14. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein

measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
15. Rannels, D. E., R. H. Sahms, and C. A. Watkins. Effects of starvation and diabetes on protein synthesis in lung. *Am. J. Physiol.* **236**:E421–E428 (1979).
16. Rannels, D. E., D. M. White, and C. A. Watkins. Rapidity of compensatory lung growth following pneumonectomy in adult rats. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* **46**:326–333 (1979).
17. Price, H. L., and R. D. Dripps. General anesthetics. II. Volatile anesthetics: diethyl ether, divinyl ether, chloroform, halothane, methoxyflurane, and other halogenated volatile anesthetics, in *The Pharmacological Basis of Therapeutics* (L. S. Goodman and A. Gilman, eds.), Ed. 4. MacMillan, New York, 79–92 (1975).
18. Rannels, D. E., E. E. McKee, and H. E. Morgan. Regulation of protein synthesis and degradation in heart and skeletal muscle, in *Biochemical Actions of Hormones*, (G. Litwack, ed.), Vol. 4. Academic Press, New York, 135–195 (1975).
19. Harris, R. A., J. Munroe, B. Farmer, K. C. Kim, and P. Jenkins. Action of halothane upon mitochondrial respiration. *Arch. Biochem. Biophys.* **142**: 435–444 (1971).
20. Seeman, P. The membrane expansion theory of anesthesia. *Prog. Anesthesiol.* **1**:243–251 (1975).
21. Trudell, J. R., and E. N. Cohen. Anesthetic-induced nerve membrane fluidity as a mechanism of anesthesia. *Prog. Anesthesiol.* **1**:315–321 (1975).
22. Richter, J. J., E. Sunderland, U. Juhl, and S. Kornguth. Extraction of mitochondrial proteins by volatile anesthetics. *Biochim. Biophys. Acta* **543**: 106–115 (1978).
23. Eyring, H., J. W. Woodbury, and J. S. D'Arrigo. A molecular mechanism of general anesthesia. *Anesthesiology* **38**:415–424 (1973).
24. Bakhle, Y. S., and Block, A. J. Effects of halothane on pulmonary inactivation of noradrenaline and prostaglandin E_2 in anesthetized dogs. *Clin. Sci. Mol. Med.* **50**:87–90 (1976).
25. Naito, H., and C. N. Gillis. Effects of halothane and nitrous oxide on removal of norepinephrine from the pulmonary circulation. *Anesthesiology* **39**:575–580 (1973).
26. Watkins, C. A., and D. E. Rannels. Effect of halothane exposure on uptake and metabolism of 5-hydroxytryptamine by rat lungs perfused *in situ*. *Fed. Proc.* **39**:367 (1980).

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