

Halothane-induced Alterations of Cyclic Nucleotide Concentrations in Three Regions of the Mouse Nervous System

Michael L. Nahrwold, M.D.,* W. David Lust, Ph.D.,† Janet V. Passonneau, Ph.D.‡

In an effort to determine whether halothane alters cyclic nucleotide levels in the nervous system, mice were exposed to air (control) or halothane 0.7, 1.4, 2.4, 3.0, or 4.4 per cent in air, for 15 minutes. After quick-freezing in liquid nitrogen, levels of 3',5'-cyclic guanosine monophosphate (cGMP) and 3',5'-cyclic adenosine monophosphate (cAMP) in the cerebral cortex, cerebellum, and spinal cord were determined. Lactate and pyruvate were measured in the cerebral cortex and cerebellum as an index of brain oxygenation, and blood-gas and pH values were measured in replicate experiments. Three groups of studies were made: 1) control, 2) low halothane concentrations (0.7-2.4 per cent) without hypoxia and acidosis, and 3) high halothane concentrations (3.0 and 4.4 per cent) accompanied by hypoxia and acidosis. Low halothane concentrations increased cGMP in the cerebral cortex, depressed it in the cerebellum, and had no effect on levels in the spinal cord. Similar alterations were seen after exposure to high halothane concentrations that included a hypoxic component, except that cGMP in the spinal cord was depressed. Since anoxia decreases cGMP in the cerebral cortex and cerebellum, the increase in cGMP in the cortex suggests that the effect of halothane cannot be attributed to hypoxia. The only effect of halothane on cAMP was to depress the levels of the nucleotide in the cortex at halothane concentrations of 2.4 per cent or more. The authors conclude that halothane has a greater effect on cGMP than on cAMP, and that the biochemical responses to the anesthetic vary among regions of the nervous system. (Key words: Anesthetics, volatile, halothane; Brain, metabolism; Metabolism, metabolites; Spinal cord.)

IT IS APPARENT that concentrations of cyclic nucleotides respond to a variety of treatments that affect the excitability of the central nervous system.¹⁻⁹ Recent neurophysiologic studies indicate that 3',5'-cyclic adenosine monophosphate (cAMP) may be involved in adrenergic and 3',5'-cyclic guanosine monophosphate (cGMP) in cholinergic transmission.¹⁰ One possible explanation for the depressant effects of anesthesia is a direct action on neurotransmis-

sion,¹¹ which may be reflected by changes in concentrations of cyclic nucleotides. Therefore, we examined the effects of halothane on both cGMP and cAMP levels in the mouse nervous system.

It has been established that the concentrations of metabolites and cyclic nucleotides in the brain are sensitive to anoxia.^{3,12} In order to distinguish between the anesthetic and hypoxic effects of halothane, blood-gas values and acid-base status were monitored during anesthesia. Adenosine triphosphate (ATP), phosphocreatine (PC), pyruvate and lactate were measured in the brain to determine whether the fixation of the tissue was adequate. Our objectives were to determine whether halothane alters cGMP or cAMP concentrations in the central nervous system of the mouse, and whether the alterations are specific to a given region of the nervous system.

Methods

National Institutes of Health general-purpose mice weighing 24 to 27 g and fed *ad lib.* were used. The animals were anesthetized in an 8-liter plastic chamber pre-equilibrated with air or halothane vaporized in air. The gas mixtures were delivered at 3 l/min with a Dräger vaporizer and a precision flowmeter. The chamber was placed in a water bath to maintain the animals' colonic temperatures at 35 to 37.5 C as determined with a thermistor probe. Inspired anesthetic concentrations were monitored by gas chromatography and expressed as percentages of a standard atmosphere. Concentrations of halothane used were 0 (control), 0.7, 1.4, 2.4, 3.0, and 4.4 per cent.

In one series of experiments, three mice were treated with each halothane concentration for 15 minutes. Heparinized anaerobic blood samples were then quickly obtained by heart puncture with a tuberculin syringe fitted with a 27-g needle and partial pressures for oxygen (P_{O₂}) and carbon dioxide (P_{CO₂}) and pH were determined with a blood-gas analyzer (Radiometer BMS 3 Mark 2). An approximation of base excess was calculated from a Siggaard-

ABBREVIATIONS

ATP = adenosine triphosphate
cAMP = 3',5'-cyclic adenosine monophosphate
cGMP = 3',5'-cyclic guanosine monophosphate
PC = phosphocreatine

* Assistant Professor and Research Director, Department of Anesthesiology, Milton S. Hershey Medical Center.

† Research Pharmacologist, Laboratory of Neurochemistry, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health.

‡ Chief, Acting, Laboratory of Neurochemistry, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health.

Received from the Anesthesia Research Laboratory, M. S. Hershey Medical Center, Hershey, Pennsylvania 17033, and the Laboratory of Neurochemistry, National Institutes of Health, Bethesda, Maryland 20014. Accepted for publication May 23, 1977.

Address reprint requests to Dr. Nahrwold: Department of Anesthesiology, University of Michigan Medical Center, Ann Arbor, Michigan 48109.

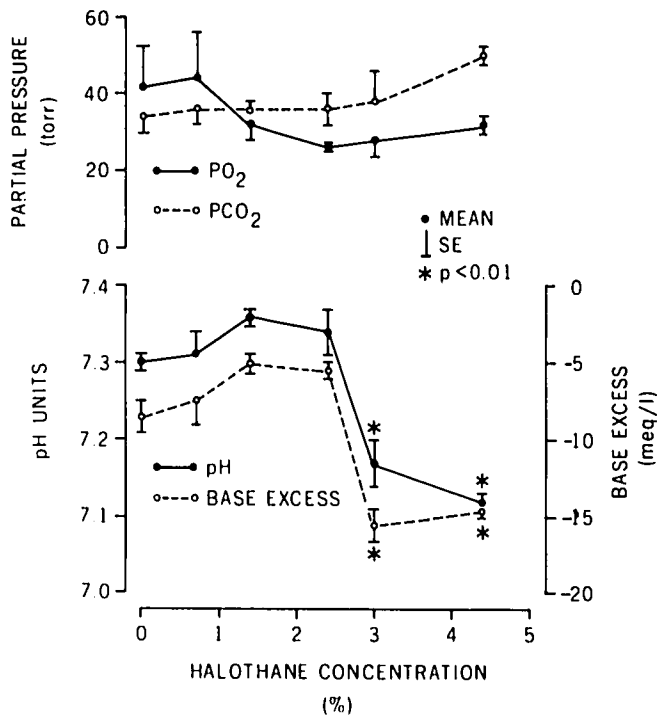


FIG. 1. Dose-response effects of halothane on P_{O_2} , P_{CO_2} , pH and base excess in the mouse. Each data point represents three animals.

Andersen nomogram assuming normal mouse hemoglobin to be 14.8 g/100 ml.¹³

For metabolite analyses, groups of five mice were exposed to halothane as described above. After 15 minutes, the mice were rapidly removed from the

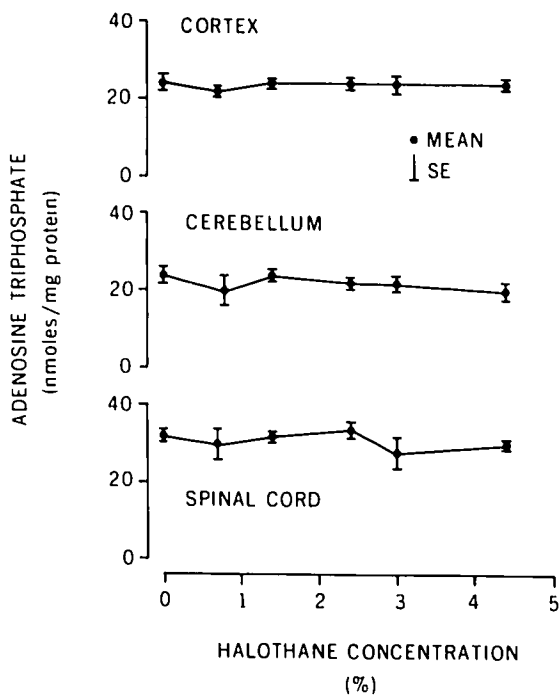


FIG. 2. Dose-response effects of halothane on adenosine triphosphate levels in mouse cerebral cortex, cerebellum and spinal cord. Each data point represents five (cerebral cortex and cerebellum) or three (spinal cord) animals.

chamber and plunged head-first into liquid nitrogen with vigorous stirring. The animals were stored at -70°C until dissection. Samples of the outer 2 mm of cerebral cortex, a wedge of cerebellum, or a small segment of the dorsal spinal cord were removed in a cryostat at -20°C . Extracts were made according to the procedures of Nelson *et al.*¹⁴ The tissues were homogenized in methanol-HCl, the protein precipitated with 0.3 N perchloric acid containing 1 mM ethyleneglycol-bis(β -aminoethyl ether) N,N' -tetracetic acid, and the homogenate centrifuged. The supernatant fluid was removed, neutralized with potassium bicarbonate, and used for the metabolite analyses. The pellet was dissolved in 1 N sodium hydroxide for measurement of protein by the Lowry method.¹⁵

The radioimmunoassay method of Steiner *et al.*¹⁶ was used to determine cAMP and cGMP in the tissue extracts. Lactate, pyruvate, ATP and PC were measured by the methods of Lowry and Passonneau.¹⁷

A dose-response plot for each of the variables under investigation was subjected to analysis of variance.¹⁸ When the results of such determinations were significant ($P < 0.05$), differences between control and experimental points were sought using Dunnett's multiple-comparison method.¹⁹

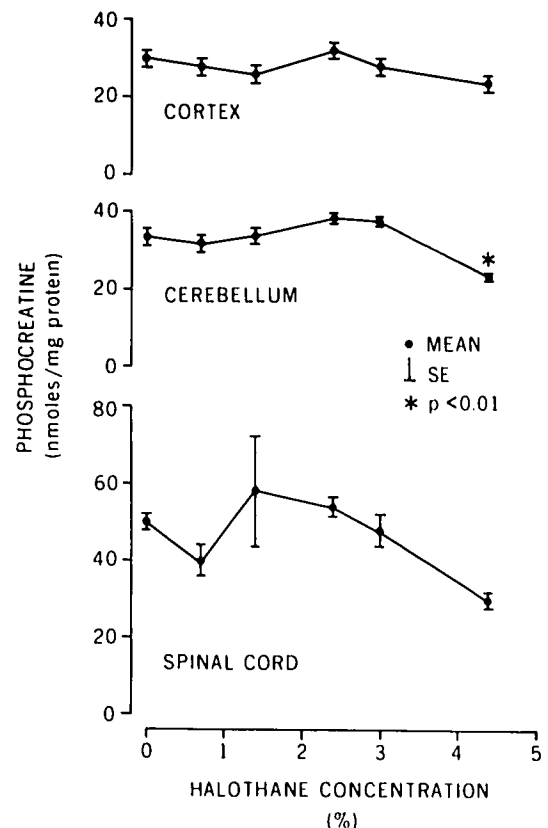


FIG. 3. Dose-response effects of halothane on phosphocreatine levels in mouse cerebral cortex, cerebellum and spinal cord. Each data point represents five (cerebral cortex and cerebellum) or three (spinal cord) animals.

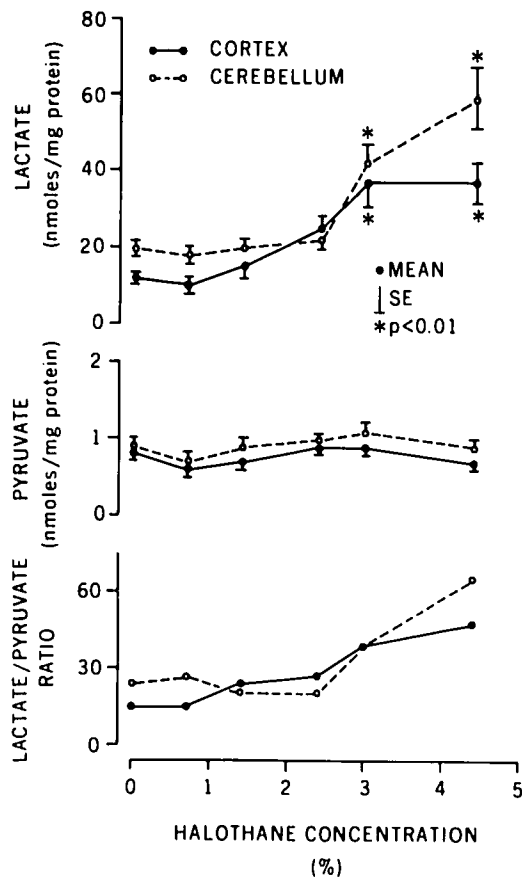


FIG. 4. Dose-response effects of halothane on lactate and pyruvate levels in mouse cerebral cortex and cerebellum. Each data point represents five animals.

Results

Mean values for P_{O_2} and P_{CO_2} did not differ significantly from control values after exposure to all concentrations of halothane, despite the fact that mice exposed to halothane, 4.4 per cent, were visibly cyanotic (fig. 1). The mixed arterial and venous nature of blood samples obtained by heart puncture may account for the lack of discernible change. Mean pH and base excess were not altered by concentrations of halothane to 2.4 per cent. However, halothane, 3.0 and 4.4 per cent, caused significant decreases in pH and base excess.

Levels of ATP and PC are known to decrease rapidly during anoxia, and the concentrations indicate to some extent the adequacy of tissue fixation.¹² Control levels of these metabolites found in the present study were comparable to those reported by other investigators using various methods of fixation (figs. 2 and 3).²⁰⁻²³ The only alteration caused by halothane was a decrease in PC levels in the cerebellum following exposure to halothane, 4.4 per cent. The results suggest that anoxia during freezing was minimal and that the metabolite concentrations approximate *in-vivo* conditions.

An indication of brain tissue oxygenation and acid-base status was obtained by measurement of lactate and pyruvate in the cerebral cortex and cerebellum. Exposure to halothane, 0.7, 1.4, or 2.4 per cent, did not affect the concentrations of either metabolite in the cerebral cortex or in the cerebellum (fig. 4). However, a significant increase in lactate was observed in both regions following treatment with halothane, 3.0 or 4.4 per cent. The pyruvate level was not observed to be altered in any animal, so lactate/pyruvate ratios mirrored the changes in lactate. These data indicate that following exposure to concentrations of halothane of 2.4 per cent or less, acid-base balance was preserved and cerebral hypoxia did not occur. However, higher concentrations of halothane resulted in hypoxia and acidosis in the cerebral cortex and cerebellum.

Control values for cyclic nucleotide levels from three regions of mouse nervous system were comparable to values reported by other investigators.⁵⁻⁹ In the cerebral cortex, cGMP levels increased to above control values following treatment with halothane concentrations of 1.4 and 2.4 per cent (fig. 5); this increase persisted during conditions of deep anesthesia. Conversely, in the cerebellum, halothane

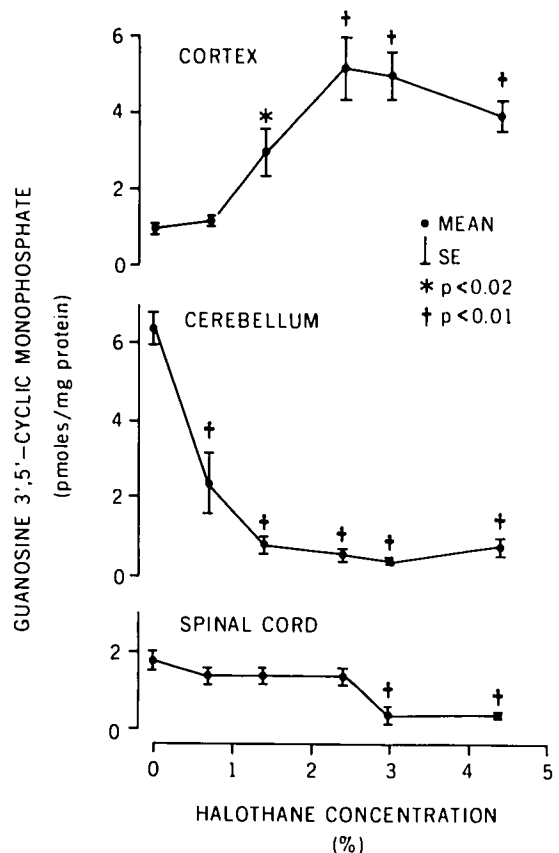


FIG. 5. Dose-response effects of halothane on cGMP levels in mouse cerebral cortex, cerebellum and spinal cord. Each data point represents five (cerebral cortex and cerebellum) or three (spinal cord) animals.

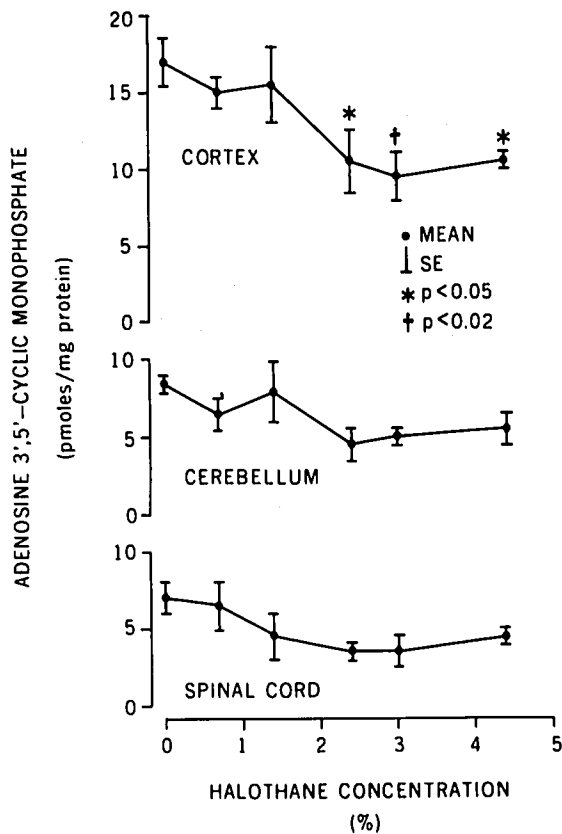


FIG. 6. Dose-response effects of halothane on cAMP levels in mouse cerebral cortex, cerebellum and spinal cord. Each data point represents five (cerebral cortex and cerebellum) or three (spinal cord) animals.

at all concentrations caused a marked decrease in cGMP levels. The levels of cGMP in spinal cord were decreased only under conditions of deep anesthesia and cerebral acidosis.

In contrast to cGMP, the concentrations of cAMP were less affected by halothane administration (fig. 6). Cyclic AMP levels were decreased only in the cerebral cortex and only after exposure to halothane concentrations of 2.4 per cent or more.

Discussion

The present investigation was designed to study the effects of a brief exposure to halothane on cyclic nucleotide concentrations in three regions of the central nervous system of the mouse. While no attempt was made to quantitate brain anesthetic tension, it has been shown that after 15 minutes of exposure to halothane, the ratio of alveolar to inspired halothane is about 0.55 in the rat.²⁴ Since anesthetic uptake is probably more rapid in the mouse,²⁴ the alveolar and brain tensions of anesthetic at the time of tissue sampling were probably half to two-thirds of the measured inspired values.

In the absence of cerebral hypoxia, halothane caused a dose-dependent increase in cGMP in the cerebral cortex. In contrast, a dose-related diminution of cGMP occurred in the cerebellum, while levels in spinal cord were unchanged. It is difficult to interpret these results because the specific biologic role of cGMP *in vivo* has not been clearly defined. However, considerable evidence has accumulated to suggest that this cyclic nucleotide may mediate cholinergic postsynaptic actions,^{4,10} possibly through regulation of the phosphorylation of specific membrane proteins.²⁵ Ferrendelli *et al.* observed increases in cGMP levels in the cortex to 290 per cent of control values following administration of the anticholinergic agent, atropine, to mice.⁸ Levels in the cerebellum were decreased to 39 per cent of control. These reciprocal changes in cGMP levels between cortex and cerebellum are nearly identical to those caused by halothane in the present investigation. Since halothane is known to increase the acetylcholine content of rat cerebral cortex slices,²⁶ perhaps alterations in cGMP levels are a reflection of interference with cholinergic mechanisms.

Anoxia caused by decapitation decreases cGMP 60 per cent in the cortex and 80 per cent in the cerebellum.⁶ Although in the present study similar effects were seen in the cerebellum during deep anesthesia and hypoxia, cGMP levels were also depressed at anesthetic concentrations that did not induce a hypoxic component. Furthermore, deep anesthesia and hypoxia increased cGMP in the cerebral cortex, suggesting that halothane exerts an effect distinct from hypoxia in this region.

Changes in cAMP levels caused by anesthesia were seen only in the cerebral cortex, where halothane, 2.4 per cent, in the absence of hypoxia caused a depression of nucleotide levels. Biebuyck *et al.* have reported a two and a half-fold increase in cAMP in rat forebrain following a 60-minute exposure to halothane, 1.5 per cent.^{1,2} The disparity between the two observations may be due to the durations of exposure to halothane, species differences, or regions of the brain sampled.

Concentrations of halothane that included a hypoxic component also decreased levels of cAMP in the cortex, an effect opposite to the fourfold increase in cAMP that occurs in response to anoxia.⁶ Both the increased cGMP and the decreased cAMP concentrations in the cortex after 15 minutes of exposure to halothane suggest an effect that differs from hypoxia.

We have observed that cGMP levels in the mouse nervous system are more readily altered by halothane than are cAMP levels. Whether this fact is of value in defining a molecular mechanism of halothane-induced narcosis remains to be seen. Certainly, the ob-

served effects of the anesthetic on cGMP levels in cerebral cortex, cerebellum, and spinal cord indicate that halothane can produce variable effects in different areas of the nervous system. As the specific biologic role of cGMP is elucidated, the implications of altered levels of this nucleotide during anesthesia can be better understood.

The authors thank Ms. Joan Wolfe for performing the blood-gas determinations.

References

1. Biebuyck JF, Dedrick DF, Scherer YD: Brain cyclic AMP and putative transmitter amino acids during anesthesia. *Molecular Mechanisms of Anesthesia*. Edited by Fink BR. New York, Raven Press, 1975, pp 451-470
2. Dedrick DF, Scherer YD, Biebuyck JF: Use of a rapid brain-sampling technique in a physiologic preparation. *ANESTHESIOLOGY* 42:651-657, 1975
3. Kimura H, Thomas E, Murad F: Effects of decapitation, ether and pentobarbital on guanosine 3',5'-phosphate levels in rat tissues. *Biochim Biophys Acta* 343:519-528, 1974
4. Goldberg ND, O'Dea RF, Haddox MK: Cyclic GMP. *Advances in Cyclic Nucleotide Research*. Volume 3. Edited by Greengard P, Robison GA. New York, Raven Press, 1973, pp 155-223
5. Kinscherf DA, Chang MM, Rubin EH, et al: Comparison of the effects of depolarizing agents and neurotransmitters on regional CNS cyclic GMP levels in various animals. *J Neurochem* 26:527-530, 1976
6. Steiner AL, Ferrendelli JA, Kipnis DM: Radioimmunoassay for cyclic nucleotides. III. Effect of ischemia, changes during development and regional distribution of adenosine 3',5'-monophosphate in mouse brain. *J Biol Chem* 247:1121-1124, 1972
7. Ferrendelli JA, Chang MM, Kinscherf DA: Elevation of cyclic GMP levels in central nervous system by excitatory and inhibitory amino acids. *J Neurochem* 22:535-540, 1974
8. Ferrendelli JA, Steiner AL, McDougal DB Jr, et al: The effect of oxotremorine and atropine on cGMP and cAMP levels in mouse cerebral cortex and cerebellum. *Biochem Biophys Res Commun* 41:1061-1067, 1970
9. Ferrendelli JA, Kinscherf DA, Chang M: Comparison of the effects of biogenic amines on cyclic GMP and cyclic AMP levels in mouse cerebellum *in vitro*. *Brain Res* 84:67-73, 1975
10. Wiklund RA: Cyclic nucleotides. *ANESTHESIOLOGY* 41:490-500, 1974
11. Halsey MJ: Mechanisms of general anesthesia. *Anesthetic Uptake and Action*. Edited by Eger EI II. Baltimore, Williams and Wilkins, 1974, pp 45-76
12. Lust WD, Passonneau JV, Veech RL: Cyclic adenosine monophosphate, metabolites, and phosphorylase in neural tissue: A comparison of methods of fixation. *Science* 181:280-282, 1973
13. *Biological Handbooks, Respiration and Circulation*. Edited by Altman PL, Dittmer DS. Bethesda, Federation of American Societies for Experimental Biology, 1971, pp 151, 222-223
14. Nelson SR, Schulz DW, Passonneau JV, et al: Control of glycogen levels in brain. *J Neurochem* 15:1271-1279, 1968
15. Lowry OH, Rosebrough NJ, Farr AL, et al: Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265-275, 1951
16. Steiner AL, Wehmann RE, Parker CW, et al: Radioimmunoassay for the measurement of cyclic nucleotides. *Advances in Cyclic Nucleotide Research*. Volume 2. Edited by Greengard P, Robison GA. New York, Raven Press, 1972, pp 51-61
17. Lowry OH, Passonneau JV: A Flexible System of Enzymatic Analysis. New York, Academic Press, 1972, pp 151-156, 194-201, 212-216
18. Batson HC: *An Introduction to Statistics in the Medical Sciences*. Minneapolis, Burgess, 1956, pp 22-37
19. Dunnett CW: A multiple comparison procedure for comparing several treatments with a control. *Am Statist Assn J* 50:1096-1121, 1955
20. Duffy TE, Nelson SR, Lowry OH: Cerebral carbohydrate metabolism during acute hypoxia and recovery. *J Neurochem* 19:959-977, 1972
21. Brunner EA, Passonneau JV, Molstad C: The effect of volatile anesthetics on levels of metabolites and on metabolic rate in brain. *J Neurochem* 18:2301-2316, 1971
22. Nahorski SR, Rogers KJ: The adenosine 3',5'-monophosphate content of brain tissue obtained by an ultra-rapid freezing technique. *Brain Res* 51:332-336, 1973
23. Lowry OH, Passonneau JV, Hasselberger FX, et al: Effect of ischemia on known substrates and cofactors of the glycolytic pathway in brain. *J Biol Chem* 239:18-30, 1964
24. Wahrenbrock EA, Eger EI II, Laravuso RB, et al: Anesthetic uptake—of mice and men (and whales). *ANESTHESIOLOGY* 40:19-23, 1974
25. Greengard P: Possible role for cyclic nucleotides and phosphorylated membrane proteins in postsynaptic action of neurotransmitters. *Nature* 260:101-108, 1976
26. Cheng S-C, Brunner EA: A neurochemical hypothesis for halothane anesthesia. *Anesth Analg (Cleve)* 54:242-246, 1975