Volume 48, No. 1
January 1978

THE JOURNAL OF Anesthesiology
THE AMERICAN SOCIETY OF ANESTHESIOLOGISTS, INC.

Editorial Views

Downloaded from http://pubs.asahq.org/anesthesiology/article-pdf/48/1/1/298137/0000542-197801000-00001.pdf by guest on 10 November 2024

Anesthesiology
48:1-3, 1978

Anesthetics and Central Cholinergic Function—A Perspective

ACETYLCHOLINE (ACh) was definitively shown to be a factor in mammalian nervous system transmission more than 50 years ago.[1,2] Although ubiquitous within the mammalian organism, it has, nevertheless, persistently eluded the attempts of investigators to clarify and completely understand factors that regulate its synthesis, release and metabolism.

This could be attributed primarily to a number of very unique characteristics of the cholinergic transmitter, which complicate attempts to study it under "natural" conditions. First, ACh is rapidly hydrolyzed by acetylcholinesterase (AChE), which is present in high concentrations in the vicinity of ACh, *in vivo*.[3,4] In order to compensate for this rapid hydrolytic effect, the cholinergic system appears under normal conditions to have tremendous regenerative powers, which maintain concentrations of this transmitter at nerve endings at a fairly constant level. Thus, levels of ACh at nerve endings "resist" perturbation under most conditions (Mann *et al.*,[5] Karlson and MacIntosh[6]; see also review of this question by Browning[7]). Second, ACh is unique among known central neurotransmitter substances in that its immediate biologic precursor, choline (Ch), is also the product of the ACh hydrolysis by the enzyme AChE. Moreover, a portion of Ch resulting from this hydrolytic process is reincorporated within the cholinergic nerve terminal and utilized for the synthesis of new molecules.[8-12]

Consequently, attempts to quantitate the rate and extent of conversion of Ch to ACh, and its subsequent metabolism in brain, were hindered until about a decade ago by the lack of the following factors: 1) efficient methods to kill the experimental animals; 2) availability of dependable, sensitive and specific methods for the analysis of levels and metabolism of Ch and its metabolites; 3) appropriate techniques with which to calculate the rate of turnover of brain ACh *in vivo*. Within the past decade, however, a number of workable solutions to the obstacles mentioned above have been found. As a result, the investigation of central cholinergic mechanisms *in vivo* has experienced an enthusiastic revival:

1) The introduction of novel techniques to kill experimental animals rapidly while inactivating endogenous enzymes within seconds, or even fractions thereof,[13-16] has considerably improved the investigator's handling of the problem of postmortem enzyme-induced changes in neurotransmitter levels in the tissues studied.

2) In the area of cholinergic research, particular stimulus has been provided by the emergence within the past ten years of a number of reliable and sensitive chemical assay techniques for the quantitative measurement of levels of Ch and ACh in very small concentrations in tissue extracts.[17] Among these techniques, the capacity of the gas chromatographic method has further been extended by attaching a fraction collector to the gas chromatographic apparatus in such a manner as to enable a portion of the effluent to be analyzed for its radioactive content, while the remainder is analyzed for quantity of the measured substance. This modification enables the analysis of specific radioactivities of the substances studied and thus is

0003-3022-78-0100—0001 $00.60 © The American Society of Anesthesiologists, Inc.

amenable to kinetic analyses and quantitation of ACh turnover rates.[11,18] Another powerful extension of the gas chromatographic method to achieve the same goal has been via its coupling with mass spectrometry and the specific technique of stable isotopic dilution and selective ion monitoring.[19-21]

3) For the analysis of turnover rates of ACh *in vivo*, several dynamic approaches that have attempted to account for the considerations of rapid regeneration of cholinergic processes and the recirculation of Ch formed in the synaptic cleft as a result of hydrolysis of ACh have been developed. Most of these approaches are relatively new and still have to stand the test of time. Nevertheless, results obtained with these approaches have contributed important information towards the understanding of the modes of action of a number of pharmacologic agents on metabolism of ACh in whole brain, as well as in specific brain areas, in experimental animals (see review by Hanin and Costa[22]).

Current knowledge regarding the role of cholinergic systems in anesthesia is relatively meager. Studies of neurotransmitter function in anesthetic states have focused mainly on the catecholamines and indoleamines. Cholinergic involvement has not received adequate attention, primarily because of the obstacles to accurate measurement of central cholinergic function *in vivo* described above. A few early studies, nevertheless, indicate that anesthetics generally depress central cholinergic activity in mammals.[23-31] Moreover, clinical evidence and studies in experimental animals[32-35] utilizing various cholinergic agonists and antagonists have demonstrated that cholinergic manipulation will affect the anesthetic process. Such information, although fragmentary and sometimes contradictory, has, at least, provided documentation for some definitive participation of ACh in the mechanism of anesthesia.

It is not surprising, therefore, that recently several laboratories that are equipped to tackle the methodologic problems described earlier have sought to determine the nature and extent of brain ACh metabolic involvement in narcosis, *in vivo*.[36-38] The paper in this issue by Ngai, Cheney and Finck is the result of one such study. Tracer kinetic analyses of ACh turnover rate *in vivo* utilizing gas chromatography have been employed following rapid inactivation of brain cholinergic enzymatic activity by microwave irradiation. Three types of anesthetics have been tested in rats: halothane, enflurane and ketamine. The expected reduction in brain ACh turnover rate was observed after administration of all three drugs. What is most important is that in the case of each drug this effect was specific for specific brain areas. The results paralleled quite remarkably the electrophysiologic effects of these selected anesthetics in cortical and subcortical structures. This work by Ngai and co-investigators, while adhering to necessary methodologic precautions and utilizing sophisticated techniques, has thus demonstrated a causal relationship between neurochemical and electrophysiologic events induced in brain by anesthetic agents.

The cholinergic system in brain, of course, does not exist as an independent neurochemical entity. One should continuously keep in mind the fact that neurotransmitters in brain, some known, many others perhaps yet to be uncovered, act in concert with one another in a complex series of relationships. Thus, the effect of anesthetics in decreasing ACh turnover rate in a particular brain area may conceivably be due to an effect on some other neurotransmitter system in the brain, which in turn induces the depressogenic effect on the particular cholinergic system. Nevertheless, one has to start somewhere, in order to be capable of building onto existing data, and thus to help unravel the intricacies of neurochemical correlates of anesthetic function.

Where will such studies eventually lead? Quite possibly to the *in vivo* analysis of the effects of anesthetic agents on brain cholinergic and other neurotransmitter-related functions in man. At present, this may seem to be a utopic and unattainable goal. Recent advances in this direction utilizing stable isotope dilution and gas chromatographic/mass spectrometric analysis are, nevertheless, quite encouraging, and show promise of yielding important information.

Experiments such as those conducted by Ngai and co-investigators in animals will provide a basis for interpretation and elucidation of experimental observations obtained by eventual studies in man.

ISRAEL HANIN, PH.D.
Associate Professor of Psychiatry
Director, Psychopharmacology Program
Western Psychiatric Institute and Clinic
Department of Psychiatry
University of Pittsburgh School of Medicine
Pittsburgh, Pennsylvania 15261

References

1. Dale HH: The action of certain esters and ethers of choline, and their relation to muscarine. J Pharmacol Exp Ther 6:147-190, 1914

Downloaded from http://pubs.asahq.org/anesthesiology/article-pdf/48/1/1/298137/0000542-197801000-00001.pdf by guest on 10 November 2024

2. Loewi O, Navratil E: Über humorale Übertragbarkeit der Herznervenwirkung. X. Mitteilung Über das Schicksal des Vagusstoffes. Pfluegers Arch Ges Physiol 214:618-688, 1926
3. Augustinsson KB: *In* Handbuch der Experimentellen Pharmakologie, XV. Edited by Koelle GB. Berlin, Springer Verlag, 1963, pp 89-128
4. Koelle GB: *In* Handbuch der Experimentellen Pharmakologie, XV. Edited by Koelle GB. Berlin, Springer Verlag, 1963, pp 187-298
5. Mann PJG, Tennenbaum M, Quastel JH: On the mechanism of acetylcholine formation *in vitro*. Biochem J 32:243-261, 1938
6. Karlson G, MacIntosh FC: Acetylcholine synthesis in a sympathetic ganglion. J Physiol (Lond) 96:277-292, 1939
7. Browning ET: Acetylcholine metabolism, Biology of Cholinergic Function. Edited by Goldberg AM, Hanin I. New York, Raven Press, 1976, pp 187-201
8. Perry WLM: Acetylcholine release in the cat's superior cervical ganglion. J Physiol 119:439-454, 1953
9. Collier B, MacIntosh FC: The source of choline for acetylcholine synthesis in a sympathetic ganglion. Can J Physiol Pharmacol 47:127-135, 1969
10. Potter LT: Synthesis, storage and release of (^{14}C) acetylcholine in isolated rat diaphragm muscles. J Physiol (Lond) 206:145-166, 1970
11. Hanin I, Massarelli R, Costa E: An approach to the *in vivo* study of acetylcholine turnover in rat salivary glands by radio gas chromatography. J Pharmacol Exp Ther 181: 10-18, 1972
12. Hanin I, Massarelli R, Costa E: An approach to the study of the biochemical pharmacology of cholinergic function, Advances in Biochemical Psychopharmacology. Edited by Costa E, Iversen LL, Paoletti R. Volume 6. New York, Raven Press, 1972, pp 181-202
13. Takahashi R, Aprison MH: Acetylcholine content of discrete areas of the brain obtained by a near-freezing method. J Neurochem 11:887-898, 1964
14. Stavinoha WB, Weintraub ST, Modak AT: The use of microwave heating to inactivate cholinesterase in the rat brain prior to analysis for acetylcholine. J Neurochem 20: 361-371, 1973
15. Veech RL, Harris RL, Veloso D, et al: Freeze blowing: A new technique for the study of brain *in vivo*. J Neurochem 20:183-188, 1973
16. Guidotti A, Cheney DL, Trabucchi M, et al: Focussed microwave radiation: A technique to minimize post mortem changes of cyclic nucleotides, DOPA and choline, and to preserve brain morphology. Neuropharmacology 13:1115-1122, 1974
17. Hanin I (ed): *In* Choline and Acetylcholine: Handbook of Chemical Assay Methods. New York, Raven Press, 1974
18. Cheney DL, Costa E, Hanin I, et al: Application of principles of steady-state kinetics in the *in vivo* estimation of acetylcholine turnover rate in mouse brain. J. Pharmacol Exp Ther 192:288-296, 1975
19. Jenden DJ, Choi L, Silverman RW, et al: Acetylcholine turnover estimation in brain by gas chromatography/mass spectrometry. Life Sci 14:55-63, 1974
20. Karlen B, Lundgren G, Nordgren I, et al: *In* Choline and Acetylcholine: Handbook of Chemical Assay Methods. Edited by Hanin I. New York, Raven Press, 1974, pp 163-179
21. Hanin I, Skinner RF: Analysis of microquantities of choline and its esters utilizing gas chromatography-chemical ionization mass spectrometry. Anal Biochem 66:568-583, 1975
22. Hanin I, Costa E: *In* Biology of Cholinergic Function. Edited by Goldberg AM, Hanin I. New York, Raven Press, 1976, pp 355-377
23. Tobias JM, Lipton MA, Sepinat AA: Effect of anesthetics and convulsants on brain acetylcholine content. Proc Soc Exp Biol Med 61:51, 1946
24. Richter D, Crossland J: Variation in acetylcholine content of the brain with physiological state. Am J Physiol 159: 247-255, 1949
25. Elliott KAC, Swank RL, Henderson N: Effects of anesthetics and convulsants on acetylcholine content in brain. Am J Physiol 162:469-474, 1950
26. Giarman NJ, Pepeu G: Drug-induced changes in brain acetylcholine. Br J Pharmacol 19:226-234, 1962
27. Mitchell JF: *In* Mechanisms of Release of Biogenic Amines. Edited by Euler USv, Rosell S, Uvnäs B. Volume 5. New York, Pergamon Press, 1966
28. Schmidt KF: Effect of halothane anesthesia on regional acetylcholine levels in the rat brain. ANESTHESIOLOGY 27: 788-792, 1966
29. Sharkawi M: Effects of morphine and pentobarbitone on acetylcholine synthesis by rat cerebral cortex. Br J Pharmacol 40:86-91, 1970
30. Nordberg A, Sundwall A: Effect of pentobarbital on endogenous acetylcholine and biotransformation of radioactive choline in different brain regions, Cholinergic Mechanisms. Edited by Waser PG. New York, Raven Press, 1975, pp 229-239
31. Modak AT, Weintraub ST, McCoy TH, et al: Use of 300-msec microwave irradiation for enzyme inactivation: A study of effects of sodium pentobarbital on acetylcholine concentration in mouse brain regions. J Pharmacol Exp Ther 197:245-252, 1976
32. Oelszner W, Ebert W, Westermann KH: Die Potenzierung der Barbitalnarkose bie Mäusen durch Cholinomimetika und Cholinesteraseblocker. Acta Biol Med Germ Band 27: 983-992, 1971
33. Albin MS, Aguirre EM: Tetrahydroaminoacridine (THA). 2. Modification of postanesthetic emergence responses and anesthesia sleep time after ketamine in man. Excerpta Med Int Congr Series #347, 1974, p 42
34. Albin MS, Bunegin L, Jannetta PJ, et al: Tetrahydroaminoacridine (THA). 1. Effect on postanesthetic emergence responses and anesthesia sleep time after ketamine, phencyclidine and thiamylal in animals. Excerpta Med Int Congr Series #347, 1974, p 41
35. Winters WD, Kott KS: Neuropharmacological interaction between ketamine and physostigmine, diazepam and propranolol. Proc West Pharmacol Soc 19:232-236, 1976
36. Domino EF, Wilson AE: Psychotropic drug influences on brain acetylcholine utilization. Psychopharmacologia 25: 291-298, 1972
37. Sundwall A: Effect of pentobarbital on the turnover of acetylcholine in brain nerve terminals *in vivo*. Brain Res 62: 531-536, 1973
38. Trabucchi M, Cheney DL, Racagni G, et al: Pentobarbital and *in vivo* turnover rate of acetylcholine in mouse brain and regions of rat brain. Pharmacol Res Commun 7: 81-94, 1975

Downloaded from http://pubs.asahq.org/anesthesiology/article-pdf/48/1/1/298137/0000542-197801000-00001.pdf by guest on 10 November 2024