A Review of Recent Studies Relating Ligand Diffusion, General Anesthesia, and Sleep

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This review article presents 3 theories related to ligand diffusion, general anesthesia and sleep. The first theory describes the diffusion of molecules across a protein surface to a receptor. It is based on the effect of the amino acid side chains on the protein surface on the structure of bulk water nearby. This influence creates pathways, called chreodes, through the water near the protein surface, permitting a rapid diffusion of molecules to the receptors.

A second theory involving the role of chreodes presents a mechanism of action of nonspecific anesthetic agents. These agents interrupt the diffusion of neurotransmitter molecules to their receptors, bringing on the anesthetic effects.

Finally, building on the similarities of anesthesia and sleep, a theory is presented proposing that an external agent influences sleep in a way similar to that of the nonspecific anesthetic molecules. This external agent is proposed to be elemental nitrogen. Several observations are presented to support this mechanism.

Keywords: Nonspecific anesthetic mechanism, protein surface water, sleep from nitrogen effect, sleep theory.

Several articles have appeared during the last 5 years presenting a new concept describing neurotransmitter molecule diffusion to a receptor,[1-3] a possible mechanism of general anesthetic action,[4] and a hypothesis of a cause of sleep.[5] These processes are dependent on water: its dynamic character, its structure, and the influence that solutes or stationary structures have on it. The novelty of this hypothesis and its broad relation to areas of our clinical focus make it a subject to review.

Liquid water is a dynamic substance, constantly changing its structure by making and breaking hydrogen bonds. Diffusion of a substance through water is a movement through the spaces (about one-third of the total volume) between clusters of hydrogen-bonded water molecules. The presence of other molecules has a varying influence on water molecules in their vicinity depending on the properties of the different species. Polar molecules (a hydrophilic property) in the solution attract water molecules into an intimate contact. Nonpolar molecules (a hydrophobic property) are not bound to water molecules, influencing local organization of water.

Chreode Theory of Molecule Diffusion

Using dynamic models, these hydrophilic and hydrophobic properties were studied to determine their effects on the water molecules immediately adjacent to a protein surface.[1-3] In a protein molecule each amino acid side chain intruding into the bulk water influences the water structure. The cavities between clusters of hydrogen-bonded water molecules are in a dynamic state, forming, joining with other cavities, breaking away, and re-forming in response to hydrogen bonding affinities. All of this is due to the influence of the hydrophilic and hydrophobic states of the amino acid side chains. These passageways, called chreodes,[1] facilitate neurotransmitter molecule diffusion through water close to the receptor protein surface. It was proposed that neurotransmitter molecules crossing a synapse encounter the receptor protein molecule and are captured within water close to the protein surface. They are then guided to the receptor through a series of water chreodes created by the influence of each amino acid side chain on the protein surface. Dynamic models used in this study supported this hypothesis.[1-3]

Several consequences of this hypothesis are evident based on pharmacodynamic behavior. One observation related to this hypothesis of chreode passage is the phenomenon of lag, the time passage before an effect of a drug molecule is observed in a pharmacological test system. The lag may reflect the time needed for a molecule to displace the normal neurotransmitter molecule from the chreodes leading to the receptor. The sequel to this observation is persistence; the measured effect from the receptor activation that continues for some time after the washout of a test molecule from the system. The presence of a neurotransmitter molecule in a chreode is proposed to resist the immediate washout process. The velocity of enzyme reactions may be explained in part by the facilitated diffusion of the substrate molecule through chreodes to the enzyme active site and the facilitated departure of the product molecule away from the active site through other chreodes.

Nonspecific Volatile Anesthetic Activity

In a subsequent article building on the chreode hypothesis,[4] the presence of the chreodes associated with each re-

ceptor on a protein surface was presented as a target for nonspecific volatile anesthetic agents. A prominent view is that weak binding, not characteristic of a specific drug-receptor encounter, occurs to produce the anesthetic effect with the volatile, nonspecific drugs. The proposed molecular mechanism of anesthesia is based on small encounters at many sites on a protein, not at the receptor on its surface. The nonspecific general anesthetic agent may act at many receptor landscapes, producing an interference of a neurotransmitter passage to their receptors. A decline in many receptor functions characterizes anesthesia.

Inhalational general anesthetic molecules are approximately the same size as those of amino acid side chains, and hydropathic states are similar to those of the hydrophobic side chains.[4] These 2 molecular properties are influential in controlling the creation of the hydrophobic effect. An anesthetic molecule in or near a chreode may alter the original pattern, disrupting the normal diffusion of the neurotransmitter to the receptor. When nonspecific molecules were modeled with the simulated chreode, they interfered with the diffusion and a slower diffusion rate occurred.[6] Because of the diversity of landscape structures associated with different receptors and the differences among the structures of the anesthetic molecules, there are some differences in the clinical profiles of each anesthetic drug. The interactions of inhaled anesthetic molecules with the chreodes are weak and nonspecific.

The patterns of the side chains forming the chreode may create local asymmetries that produce different responses to chiral isomers of an anesthetic agent. Another recent study has modeled segments of chreodes using cellular automata dynamics.[6] When the diffusion behavior of chiral molecules was compared in the model, there were modest differences in the rate of diffusion among stereoisomers. This corresponds to the stereospecificity observed with some general anesthetics.[7]

In summary, the presence of an inhalation anesthetic drug in a chreode system supporting a neurotransmitter diffusion to a receptor was proposed to influence the structure of the chreodes, each in a characteristic way depending on their molecular structures. This leads to the altered function of the chreode, thereby reducing the diffusion and the receptor response. The summation of these numerous diffusion disruption events was proposed to produce clinical anesthesia.

Relation Between General Anesthesia and Sleep

It is believed that multiple receptors must be involved for anesthetic action to account for the variety of observed effects.[8-11] General anesthesia and sleep have some remarkably similar physiological and behavioral effects.[12] There is strong support for the hypothesis that neuronal networks that regulate natural sleep are involved in sedation and anesthesia.[8,13,14] Sleep and anesthesia eliminate wakefulness via many brain regions.[15] Among these the pons has become a focus of study of arousal states.[16-21] Cholinergic neurotransmission in the pons is a causal factor in arousal state control.[22-24]

The ideal anesthetic state is a composite of reversible characteristics including analgesia, amnesia, unconsciousness, and skeletal muscle relaxation.[25] Normal sleep also requires the timed coordination of these characteristics.[26] General anesthetics produce the unconscious state by, in part, interfering with central nervous system (CNS) cholinergic neurotransmission.[27-31] Cortical acetylcholine release is greater during wakefulness and rapid eye movement (REM) sleep than during non-REM sleep and anesthesia.[32-34] Cholinergic brainstem neurons produce an activated cortical electroencephalogram (EEG) during wakefulness. During non-REM sleep and general anesthesia there is a decrease in pontine cholinergic neurotransmission and a deactivated cortical EEG. The spindles in the EEG for halothane have the same appearance and frequency as the spindles for non-REM sleep.[35-37] These observations led to the conclusion that the mechanism of both sleep and anesthesia is an inhibition located in neurotransmission systems, prominently CNS cholinergic, leading to a loss in the waking state. If the mechanism is the same, then the possibility arises that sleep may be invoked by an exogenous source, just as is general anesthesia.

A Theory of Sleep

The chreode model of neurotransmitter molecule diffusion and its possible role in the mechanism of general anesthesia led to the consideration of the closely related natural phenomenon of sleep.[5] A recent review describes what is currently known about anesthesia and sleep, recognizing that there is a significant similarity between these 2 processes.[26] Anesthesia has a primal event, the introduction of an exogenous agent. From the water chreode theory of general anesthesia, this is a nonspecific chreode-modifying drug, a general anesthetic.

A recently proposed hypothesis of the origin of sleep was based on the water chreode theory and its role in the mechanism of anesthesia.[5] It was based on the hypothesis that sleep is due to an exogenous substance, sometimes referred to as a sleep factor. The mechanism is the same as in anesthesia—various chreodes associated with receptors in the CNS and elsewhere are interfered with. These receptors function in many ways in neurotransmission and reuptake and are affected in varying degrees, producing a pattern of physiological responses collectively called sleep.

What is this exogenous substance leading to sleep? It was proposed that it is nitrogen.[5] Nitrogen makes up 78% of the air we breathe, drawn in with oxygen, which is essential for life. Nitrogen is inert; current studies reveal that it plays no role in any life process, but all terrestrial

life has evolved in the presence of nitrogen. Nitrogen is taken into the body with each breath and is distributed throughout like a nonspecific, volatile anesthetic drug. While a person is awake, there is an increasing accumulation of nitrogen in tissues. At some point there is enough nitrogen to interfere with a wide variety of chreodes, causing some decline in their function. This is the onset of sleepiness, ultimately leading to sleep. During sleep, intermediate states occur as a result of variations in the level of the nitrogen overburden. The nitrogen level is influenced by the slower respiration rate during sleep. Sleep produces a net reduction in the accumulated nitrogen concentration, producing a lessened effect on the chreodes, ultimately allowing a return to a waking state. The chreode targets of the nitrogen may be associated with several neurotransmitters in the CNS that are important to the sleep state, and they also may be a cascade of receptors throughout the body.

Nitrogen is known to produce anesthesia and is well known as the cause of the deep diving condition of nitrogen narcosis. Trudell and colleagues[38,39] have studied the anesthetic effect of noble gases and hydrogen and nitrogen. The common features among these gases is their chemical inertness, approximately spherical shape, and their relatively small size. The gases, hydrogen, helium, and neon are not reported to be anesthetic, but argon, krypton, xenon, and nitrogen are. Trudell and colleagues concluded that the anesthetic effect was a chemically nonspecific encounter at certain sites in the living system. The minimum alveolar concentration of the 4 anesthetic gases showed that the value for nitrogen was the highest, indicating a low level of potency. Thus, at 1 atmosphere there is no immediate anesthesia or sleep produced. An accumulation over a considerable period of time would be the only way to account for an influence on sleep.

Sleep-deprived individuals require less volatile anesthetic to produce anesthesia or to potentiate the onset and duration of isoflurane anesthesia.[40] Sleep deprivation is hypothesized to be an excess accumulation of nitrogen, resulting in a smaller amount of anesthetic drug required to create the conditions for chreode disruption. Nitrogen was studied for its effect on the decrease in sodium channel currents because of increases in the percentage of channels in the inactive state.[41] The results were the same as with volatile general anesthetics.[42,43] Abraini[44] has reported that some of the mechanisms of nitrogen and argon narcotic action appear to be similar to those of clinical inhaled anesthetics.

In these articles,[1-5] a mechanism focusing on diffusion of agents through water is described and proposed as the event leading to receptor function, general anesthesia, and sleep. Sleep is hypothesized to be an emergent property of a complex series of modifications of systems that function normally in the waking state. This hypothesis of the role of nitrogen as the exogenous sleep factor is compatible with many observations and with the complex pattern of endogenous events currently recognized.

REFERENCES

1. Kier LB, Cheng CK, Testa B. A cellular automata model of ligand passage over a protein hydrodynamic landscape. *J Theor Biol.* 2002; 215(4):415-426.
2. Ghaemi N, Rezaei-Ghaleh N, Sarbolouki MN. Directed ligand passage over the surface of diffusion-controlled enzymes: a cellular automata model. *Lecture Notes Comput Sci.* 2004;3305:719-724.
3. Marashi SA, Behrouzi R. Modeling directed ligand passage toward enzyme active site by a double cellular automata model. *Biochem Biophys Res Commun.* 2005;333(1):1-4.
4. Kier LB. A theory of inhaled anesthetic action by disruption of ligand diffusion chreodes. *AANA J.* 2003;71(6):422-428.
5. Kier LB. Water as a complex system: its role in ligand diffusion, general anethesia, and sleep. *Chem and Biodivers.* 2007;4(10):2473-2479.
6. Kier LB, Cheng CK, Testa B. Studies of ligand diffusion pathways over a protein surface. *J Chem Inf Comput Sci.* 2003;43(1):255-258.
7. Franks NP, Lieb WR. Stereospecific effects of inhalation general anesthetic optical isomers on nerve ion channels. *Science.* 1991;254 (5030),427-430.
8. Lydic R, Baghdoyan R. Cholinergic contributions to the control of consciousness. In: Yaksh T, Lynch C, Zapol WM, Maze M, Biebuyck JF, Saidman IJ, eds. *Anesthesia: Biological Foundations.* New York, NY: Lippencott-Raven Press; 1998,435-450.
9. Sawamura S, Kingery WS, Davies MF, et al. Antinociceptive action of nitrous oxide is mediated by stimulation of noradrenergic neurons in the brainstem and activation of [alpha] 2B adrenoceptors. *J Neurosci.* 2000;20(24):9242-9251.
10. Urban BW. Current assessment of targets and theories of anaesthesia. *Br J Anaesth.* 2002;89(1):167-183.
11. Campagna JA, Miller KW, Forman SA. Mechanisms of inhaled anesthetics. *N Engl J Med.* 2003;348(21):2110-2124.
12. Lydic R. Fact and fantasy about sleep and anesthesiology. *Anesthesiology.* 2002;97(5):1050-1051.
13. Lydic R, Biebuyck JF. Sleep neurobiology: relevance for mechanistic studies of anaesthesia. *Br J Anaesth.* 1994;72:506-508.
14. Lydic R. Reticular modulation of breathing during sleep and anaesthesia. *Curr Opin Pulm Med.* 1996;2(6):474-481.
15. Lydic R, Baghdoyan HA. *Handbook of Behavioral State Control: Cellular and Molecular Mechanisms.* Boca Raton, FL: CRC Press; 1999;
16. Webster HH, Jones BE. Neurotoxic lesions of the dorsolateral pontomesencephalic tegmentum-cholinergic cell area in the cat: II. effects upon sleep and waking states. *Brain Res.* 1988;458(2):285-302.
17. Lavie P, Pratt H, Scharf B, Peled R, Brown J. Localized pontine lesion: nearly total absence of REM sleep. *Neurology.* 1984;34(1):118-120.
18. Gironell A, de la Calzada MD, Sagales T, Barraquer-Bordas L. Absence of REM sleep and altered non-REM sleep caused by haematoma in the pontine tegmentum. *J Neurol Neurosurg Psychiatry.* 1995;59(2):195-196.
19. Kimura K, Tachibana N, Kohyama J, Otsuka Y, Fukozawa S, Waki R. A discrete pontine ischemic lesion could cause REM sleep behavior disorder. *Neurology.* 2000;55(6):894-895.
20. Autret A, Laffont F, de Toffol B, Cathala HP. A syndrome of REM and non-REM sleep reduction and lateral gaze paresis after medial tegumental pontine stroke: computed tomographic scans and anatomical correlations in four patients. *Arch Neurol.* 1988;45(11):1236-1242.
21. Kushida CA, Rye DB, Nummy D, Milton JG, Spire JP, Rechtscaffen A. Cortical asymmetry of REM sleep EEG following unilateral pontine hemmorrhage. *Neurology.* 1991;41(4):598-601.
22. Domino EF, Yamamoto K, Dren AT. Role of cholinergic mechanisms in states of wakefulness and sleep. *Prog Brain Res.* 1968;28:113-133.
23. Sitaram N, Wyatt RJ, Dawson S, Gillin JC. REM sleep induction by physostigmine infusion during sleep. *Science.* 1976;191(4233):1281-1283.
24. Sitaram N, Moore AM, Gillin JC. Experimental acceleration and slow-

ing of REM sleep ultradian rhythm by cholinergic agonist and antagonist. *Nature.* 1978;274(5670):490-492.

25. Trevor AJ, Miller RD. Relevance of Anesthesiology for Sleep Medicine. In: Katsung BG, Ed. *General Anesthetics, Basic and Clinical Pharmacology.* Norwalk, CT: Appleton & Lange; 2001: 419-435.
26. Lydic R, Baghdoyan HA. Sleep, anesthesiology, and the neurobiology of arousal state control. *Anesthesiology.* 2005;10:1268-1295.
27. Richards CD. In search of the mechanism of anesthesia. *Trends Neurosci.* 1980;1(3):13-16.
28. Meuret P, Bachman SB, Bonhomme V, Plourde G, Fiset P. Physostigmine reverses propofol-induced unconsciousness and attenuation of the auditory steady state response and bispectral index in human volunteers. *Anesthesiology.* 2000;93(3):708-19.
29. Lambert DG, Appadu BL. Muscarinic receptor subtypes: do they have a place in clinical anesthesia? *Br J Anesth.* 1995;74:497-499.
30. Durieux ME. Muscarinic signaling in the central nervous system: recent developments and anesthetic implications. *Anesthesiology.* 1996;84(1):173-189.
31. Perry E, Walker M, Grace J, Perry R. Acetylcholine in mind: A neurotransmitter correlate of consciousness? *Trends Neurosci.* 1999;22(6): 273-280.
32. Celesia GG, Jasper HH. Acetylcholine released from cerebral cortex in relation to state activation. *Neurology.* 1966;16(11):1053-1063.
33. Jasper HH, Tessier J. Acetylcholine liberation from cerebral cortex during paradoxical sleep. *Science.* 1971;172(983):601-602.
34. Marrosu F, Portas C, Mascia MS, et al. Microdialysis measurement of cortical and hippocampal acetylcholine release during sleep-wake cycle in freely moving cats. *Brain Res.* 1995;671(2):329-332.
35. Steriade M, Timofeev, I. Neuronal plasticity in thalamocortical networks during sleep and waking oscillations. *Neuron.* 2003;37(4):563-576.
36. Steriade M, Contreras D, Curro'Dossi R, Nunez A. The slow (<1 Hz) oscillation in reticular thalamic and thalamocortical neurons: scenario of sleep rhythm generation in interacting thalamic and neocortical networks. *J Neurosci.* 1993;13(8):3284-3299.
37. Steriade M. Cholinergic blockage of network and intrinsically generated slow oscillations promotes waking and REM sleep activity patterns in thalamic and cortical neurons. *Prog Brain Res.* 1993;98:345-355.
38. Trudell J, Koblin DD, Eger EI. A molecular description of how noble gases and nitrogen bind to a model site of anesthetic action. *Anesth Analg.* 1998;87(2):411-418.
39. Koblin DD, Fang Z, Eger EI, et al. Minimum alveolar concentrations of noble gases, nitrogen and sulfur hexafluoride in rats: helium and neon as nonimmobilizers (nonanesthetics), *Anesth Analg.* 1998;87(2): 419-424.
40. Tung A, Szafran MJ, Bluhm B, Mendelson WB. Sleep deprivation potentiates the onset and duration of loss of righting reflex induced by propofol and isoflurane. *Anesthesiology.* 2002;97(4):906-911.
41. Kendig JJ. Nitrogen narcosis and pressure reversal of anesthetic effects in the node of Ranvier. *Am J Physiol.* 1984;246(1, pt 1):C91-C95.
42. Bean BP, Shrager P, Goldstein DA. Modification of sodium and potassium channel gating kinetics by ether and halothane. *J Gen Physiol.* 1981;77(3):233-253.
43. Kendig JJ, Courtney KR, Cohen EN. Anesthetics: molecular correlates of voltage and frequency-dependent sodium channel block in nerve. *J Pharmacol Exp Ther.* 1979;210(3):446-452.
44. Abraini JH, Kriem B, Balon N, Rostain JC, Risso JJ. Gamma-aminobutyric acid neuropharmacological investigations on narcosis produced by nitrogen, argon, or nitrous oxide. *Anesth. Analg.* 2003;96(3):746-749.

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