Biochemical Action of Anesthesia Revealed

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Anesthesia, the term was coined by Oliver Wendell Holmes in 1846, to portray insensibility to pain induced by some drug, shortly after the demonstration of loss of consciousness by inhaled *ether* during a surgical procedure. General anesthesia, for almost two centuries (175 years), has made scientists wonder that how under its influence a person loses consciousness and his body becomes insensitive to pain yet the body continues performing normal vital physiologic functions. This clearly demonstrates that the central nervous system (CNS) is their primary site of action, where the nerve transmission is reduced at the synapses which release the neurotransmitters for action in the body. The only information researchers had was that general anesthesia works by suspending signals from the brain and body and blocks any record of pain or event during this period.

General anesthetic agents, administered through breathing are a collection of various chemically volatile hydrophobic molecules and most commonly used are lipid-soluble and structurally related to *ether*. Since their route of administration, they are called inhalational or volatile anesthetics. Since the exact mechanism of action was not fully clear, scientists hypothesized they act by blocking the ion channels and proteins of the neurotransmitters, by interacting with these proteins in a lipid environment.

The action of local anesthetics, is however quite clear that they take up a different mechanism employing a different class of pharmacologic agents, such as Novocain. By binding to sodium ion channel in the cell membrane of nerve cells it inhibits its function by blocking the nerve transmission to pain centers in the central nervous system. The movement of nerve impulse around the site of injection is obstructed by this action; nonetheless, no change in other areas in sense perception or awareness is observed.

Regarding general anesthesia, the breakthrough came from Research published in PNAS, "*Studies on the mechanism of general anesthesia*," on May 29, 2020. The researchers Richard Lerner, MD, and molecular biologist Scott Hansen, resolved the century-old scientific enigma through the discovery of the mechanism of action of anesthesia[1]. The success was achieved using direct stochastic optical reconstruction microscopy (dSTORM), an innovation in microscope technology.

Advances in Super-resolution techniques *have been accomplished through utilizing many techniques such as photon tunneling microscopy,* near field scanning optical microscopy and Pendry Superlens, all aided by computer-assisted methods such as detector-based pixel reassignment (e.g., re-scan microscopy[2], deconvolution[3] or pixel reassignment[4], the 4Pi laser scanning fluorescence microscope, and structured Vertico spatially modulated illumination (Vertico-SMI) microscopy technologies. Stochastic optical reconstruction microscopy (STORM), is a super-resolution imaging technique that sequentially activates and localizes photoswitchable fluorophores by time-resolved localization building higher resolution images. Fluorophores are activated to a fluorescent state during imaging. A super-resolution image is constructed from the image data produced by repeating the process allowing numerous fluorophores to accumulate. STORM uses dyes, Cy5 and Cy3, to attach to nucleic acids or proteins with a wide range of probes and labeling strategies. The three-dimensional imaging by STORM uses optical astigmatism for axis positions (x, y and z) for up to several micrometers thickness samples even in living cells. Eric Betzig, W.E. Moerner and Stefan Hell, in 2014 received Nobel Prize in Chemistry for developing this super-resolved fluorescence microscopy.

Lerner and Hansen, using nanoscale microscopic techniques, in living cells and fruit flies, demonstrated clusters of lipids as a missing go-between in a two-part mechanism in the cell membrane serve. Exposure to anesthesia temporarily converts the ordered state of lipid clusters to move into a disordered state,

increasing their diameter and GM1 (monosialotetrahexosyl ganglioside) or the area of cell membrane lipid clusters, and then reverse again, subsequently causing multiple effects which are the basis of changes in consciousness. They used (chloroform and isoflurane) to activate an anesthetic-sensitive TREK-1, a two-pore-domain potassium (K2P) channel which is activated by phospholipase D2 (PLD2). The tightly organized packed ball GM1 cluster is converted into a disordered cluster through disruption of fluorescent-tagged PLD2.

Watching tagged PLD2 via the dSTORM microscope, the researchers were able to observe PLD2 molecules scatter like billiard balls, over to a different, less-preferred lipid cluster called PIP2, away from its GM1 home. These PIP2 clusters are activated key molecules in TREK1 potassium ion channels and phosphatidic acid (PA), their lipid activator.

TREK-1 channels also called TWIK-related K+ channels, present all through the central nervous system are stimulated by polyunsaturated fatty acids and lysophospholipids and are inhibited by neurotransmitters by increasing intracellular cAMP which activates the Gq protein pathway[5]. General anesthetic agents, including diethyl ether, chloroform, propofol, isoflurane and xenon, activate TREK-1 by disrupting phospholipase D2 (PLD2) localizing lipid rafts and ultimately producing lipid phosphatidic acid (PA). Dead PLD2 vigorously blocks anesthetic TREK-1 currents in whole-cell patch-clamp recordings. In short, TREK1 under the influence of anesthetics, release potassium, hyper-polarizes the nerves and just shuts it down, losing its ability to fire, leading to the state of unconsciousness, the researchers explained. To validate their findings in a living animal model, the researchers used PLD expression knocked out fruit fly, *Drosophila melanogaster*. They found these knocked-out flies resistant to anesthetics, requiring double exposure to get the equivalent sedation effect from anesthetics.

Researchers believe that this will unravel molecular events of the brain's other hidden mysteries such as falling asleep and waking up. There are, however, many questions still unanswered. This may not be the only pathway in charge of the anesthetic activity, researchers agree. Why some people under anesthesia do not wake up at all? What are the key mediators or proteins in other words of anesthesia?

Researchers still cannot fully describe the two complicating factors in the action of these agents. Firstly, volatile anesthetics require high concentrations for action which mediate many of the nerve cell membrane protein's functions. This makes it difficult for the researcher to determine the key anesthetic action mediator. Secondly, volatile anesthetics primarily interact with proteins in a lipid environment for their effect on synaptic neurotransmission. Structural data of these proteins is required to understand the interaction of anesthetics with proteins and how their function is altered. It is difficult to detect the structural information of membrane proteins. With the absence of data in this regards it is not possible to determine the point of the primary effect of anesthetics whether it is by directly interacting with these proteins, or in some way through interaction with the surrounding lipids. Thus, scientists are exploring modeling of anesthetic binding to protein, a different approach using the sophisticated model of structural proteins in a lipid environment and determination of the detailed structure of anesthetic binding to soluble proteins to explain anesthetic action at the molecular level.

These limitations, however, did not discourage the researchers. The induction of an anesthetic "state" at the molecular level is being explored by using multiple techniques in molecular biology. Pharmacologic agents, ranging from single atoms to a wide range of anesthetics can produce loss of awareness and insensibility to pain, for example, xenon to polycyclic hydrocarbons. All these different agents do not appear to have the same molecular target. Therefore, the concept that all anesthetic agents act through one molecular mechanism of action is a most likely generalization. Molecular genetics techniques are now also being utilized to study the mechanism of action of anesthesia. So far researchers, in lower organisms, have received promising results by altering specific protein function, they have detected the protein link-to-resistance to anesthetic action. This research can help identify the specific proteins to target for anesthetic action.

Today, the advances in technology provide us an opportunity to utilize these tools to answer many obligatory questions. Future research with new insights into this area will benefit humanity by fulfilling the gaps in knowledge.

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