

Exploring the mechanisms of general anesthesia

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1. INTRODUCTION

For more than a half-century, anesthetics have been used in the clinical setting whilst their mechanisms of action on the brain remained unknown. The lipid theories of anesthesia (Meyer Overton rule) claimed that anesthetics produced loss of consciousness via direct interactions with the lipid bilayers of the neuronal membranes. This erroneous view of the mechanisms of anesthesia has really changed once sophisticated, powerful, neuroscience investigation techniques became available. These techniques (patch-clamp, genetically modified animals, proteomics and brain imaging) allowed to perform a decisive step forward in the understanding of the subtle molecular interactions of anesthetics with their protein targets (1). It is well accepted now that anesthetics produce their effects on the CNS by modulating the activity of ligand- and/or gated ionic channels located on the neuronal membranes in the CNS. However, the mechanisms of anesthetic actions are complex, and considering only one ionic channel as the unique relevant target of anesthetics cannot account for the reported effects of these agents in the brain (2, 3). Indeed, anesthetics produce multiple desirable (anxiolysis, amnesia, loss of consciousness, immobility) and undesirable (respiratory depression, hyperexcitability and convulsions, hemodynamic instability, postoperative nausea and vomiting) short term effects. Interestingly, there is accumulating experimental evidence that anesthetics also affect brain functions on the long term, both in a desirable (preconditioning, neuroprotection) and maybe undesirable (neurotoxicity, postoperative cognitive dysfunction, sleep-wakefulness disorders) with particular sensitivity of the elder brain. These long term actions of anesthetics are likely to be mediated by interference with cellular signaling going from DNA transcription into RNA to the post-transcriptional regulation of protein activity by phosphorylation. Finally, brain imaging techniques such as fMRI and PETscan indicate that anesthetic-actions on the brain such as loss of consciousness or amnesia are mediated by changing the balance activity of

specific neuronal pathways /networks that normally anatomically and functionally connect important brain areas to each other. In this brief review, we will first examine the molecular characteristics of the hypnotic, immobilizing and amnesic actions of anesthetics. We will briefly comment on the changes in specific neuronal pathways induced by anesthetics that may account for some of their effects on the brain. Finally, we will discuss the molecular bases supporting long term effects of anesthetics on the brain.

2. THE RECEPTORS AND CHANNELS, CELLULAR SIGNALING PATHWAYS AND BRAIN AREAS INVOLVED IN SHORT TERM EFFECTS OF ANESTHETICS

2.1. *Loss of consciousness*

A good candidate as a molecular target of anesthetic action in the brain must fulfil the following pharmacodynamic criteria (3): 1. Clinical concentrations of the agent less induces reversible changes in target function. 2. The expression of the target is observed in brain areas likely to be involved in the expected effect of the agent (unconsciousness, amnesia, immobility). 3. Stereoselectivity of the effects is observed in vivo and in vitro 4. The target exhibits sensitivity to anesthetics, but not to structurally close nonanesthetics Only a restricted number of molecular targets comply with these requirements. Elegant studies performed both in vivo and in vitro using a combination of patch-clamp, targeted mutations of the GABA-A receptors and behavioral experiments have shown that

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activation of the GABA-A receptor-coupled chloride channels was the best candidate to account for the hypnotic action of anesthetics. In fact, these receptors are present in almost all brain cortical and subcortical areas and are the targets of the major inhibitory neurotransmitter in the brain by combination of at least 19 subunits (4). Both etomidate and propofol induce their hypnotic effects via activation of the GABA-A receptors. Almost all anesthetics interact with this receptor by allosterically modulating the opening probability of the chloride channel inside the receptor in the presence of GABA, the natural ligand. Things are more complex than expected, since propofol is also able to directly open these channels in the absence of GABA, which suggests the possibility of two different binding sites (1). Xenon and ketamine are remarkable exceptions to this rule, since they produce their hypnotic effects by blocking both the AMPA and NMDA glutamate receptors (5, 6). Dexmedetomidine has a specific mechanism of action consisting of the activation of α_2 A-adrenoceptors, which is coupled to adenylate cyclase via a Gi protein. Activation of these receptors in the locus ceruleus result in a decrease in the firing of noradrenergic neurons located in this structure (7). Targeted mutations have shown that these agent exquisitely bind to a specific site located between the second and third transmembrane domain of the GABA-A receptor. Volatile anesthetics also act as GABA-A receptor agonists with additive effects when combined together or with intravenous agents (8,9). Substitution of the α 1-Ser-270 residue of the second transmembrane domain and of the α 1-Ala-291 residue of the third one result in the loss of hypnotic effect of volatile anesthetics, while such substitutions of the β subunit alter propofol hypnotic action (1). Anesthetics first bind the pocket site which results in stabilization of the open state of the chloride channel and gating. Several anesthetic molecules can bind to the same site of the receptor by presenting different spatial orientations (1-3). Additivity of propofol and sevoflurane actions on the GABA-A receptor are consistent with this hypothesis (8). Volatile anesthetics also increase the activity of K channels, such as TREK1 (2P channels), which results in membrane hyperpolarization, reduction in the magnitude of excitatory responses and alteration of the synchronism of neuronal (1). The cAMP-regulated pace maker HCN 1h channels are blocked by volatiles and some intravenous agents and represent other potential targets. The role of voltage-gated Na channels in production of loss of consciousness has been recently revisited

(10). Isoflurane non linearly reduces the magnitude of excitatory postsynaptic potentials and exocytosis via a decrease in the activity of these channels in the calyx of Held. This action does not depend on the presence voltage-gated Ca channels and results in a phasic inhibition of neuronal activity in the appropriate cortical and subcortical brain areas.

Neocortical circuitry appears crucial in inducing hypnosis by anesthetics because of the presence of the GABA-A receptors in most of these areas (11). Anesthetics may disrupt the balance in some neuronal networks involved in generation of NREM sleep: this is particularly the case for the locus ceruleus-hypothalamic-cortical circuit (7, 12). Disruption of this balance may occur by shutting off the activity of the noradrenergic neurons via stimulation of the α_2 -adrenoceptors of the locus ceruleus (this is the case for dexmedetomidine), or at the tuberomammillary nucleus for anesthetics that behave as GABA-A receptor agonists (13-15).

2.2. Amnesia

Anterograde amnesia is a major property of anesthetic agents. The two major limbic structures involved in the encoding of memory are the hippocampus and the amygdala (16, 17). The hippocampus is involved in context-dependent conscious memory, whilst the amygdala is responsible of fear-conditioned and emotional memory. Recent elegant brain imaging investigations have recently confirmed these hypotheses (18, 19). These actions involve both GABA-A and glutamate-mediated neurotransmissions. Isoflurane blocks LTP formation in the hippocampus (17). Also, extrasynaptic GABA-A receptors exhibit an exquisite sensitivity to very low concentrations of anesthetics and they are abundant in the hippocampus. Low concentrations of GABA result in a tonic activation of these receptors, which is enhanced by anesthetics. This mechanisms may account in part for the amnestic effects of anesthetics (3, 20). Interestingly, the α 5-subunit of the GABA-A receptor seems to play a prominent role as a target for the amnestic effects of anesthetics, since mice carrying deletion of this subunit are genetically resistant to anesthetic-induced amnesia. In contrast, some brain areas such as the prefrontal cortex are still active when propofol induces amnesia (21). Recently, Alkire and colleagues have shown using brain imaging that very low concentrations of sevoflurane could block the emotional memory in volunteers by decreasing the activity of the amygdala, and that this effect was perceptible until 8 days after anesthesia (22). There

is also some evidence that hippocampal and cortical connectivity is changed during the anesthetic state, and that some of these changes may be involved in anesthetic-induced amnesia (13).

2.3. Immobility

Immobility is a fundamental property of anesthetics. Activation of GABA-A receptors by etomidate and propofol produce immobility in the mouse (4). Recent data indicate that immobility is mediated via spinal, rather than supraspinal, mechanisms (23). The understanding of the relationships between the electrophysiological and molecular effects of anesthetics at the spinal cord level on identified targets such as GABA-A receptors, glycine receptors, NMDA receptors TREK-1 channels, remains to be established (24). It has been suggested that the spinal GABA-A receptors involved in anesthetic-induced immobility may exclusively consist of the $\beta 3$ subunit (2).

Also, pharmacogenetic data suggest individual sensitivity to anesthetics in humans, by showing that the polymorphism of the gene encoding for THF is directly related to homocysteine production induced by nitrous oxide (25).

3. CELLULAR AND MOLECULAR MECHANISMS INVOLVED IN POSSIBLE LONG TERM EFFECTS OF ANESTHETICS

Beyond their actions at the membrane receptor levels, there is growing evidence that anesthetics also interfere with cellular signaling. Some of these targets may account in part of long term effects of anesthetics on brain protection, neurotoxicity, and perhaps postoperative cognitive dysfunction in the elderly brain.

3.1. Effects of anesthetics on gene expression

Characterization (concentration, activity) of proteins present in a biological sample is called proteomics. Genetic control of protein expression can be modulated by several phenomena including chromatin remodeling, transcription, maturation and translation of RNA into proteins, post-transcriptional modifications by phosphorylation or degradation. In addition to their short-term effects induced by modulation ionic channel/receptors, anesthetics modulate protein expression. We have shown that anesthetics stimulate phosphorylation of key signalling enzymes such as the FAK tyrosine kinase (26). These nonreceptor tyrosine kinases are

good candidates to account for coupling of rapid phenomena such as depolarization or neurotransmitter release to long-lasting changes in plasticity and survival. Microarrays have allowed to characterize the quantity and functional activity of proteins present in a biological sample (27). A number of pioneer studies have examined the changes induced in protein expression of a part of the genome by a short exposure to anesthetics. In one of them, the entire genome has been examined in the basolateral amygdala. A short exposure to isoflurane (1.4 MAC, 15 min) induced long lasting changes in the expression of 269 genes involved in functionally important processes (28). These genes were linked to DNA transcription, protein synthesis and cytoskeletal protein. It can be speculated that if these effects were only partially reversible (i.e. in the aging brain), they could contribute to postoperative cognitive dysfunction in elderly surgical patients. The same consideration may apply to perturbations of the sleep-wakefulness cycles that develop in the hours and following anesthesia and surgery (29).

3.2. Neuroprotection and preconditioning

Considerable progress has been made in the understanding of the consequences of anoxia/ischemia on the brain. Noteworthy, increase in cellular Ca concentration plays a key role in the development of cell death by necrosis and apoptosis by favoring the production of ROS, alteration of DNA synthesis and activation of proteolytic enzymes. Regulatory proteins such as the Heat Shock Proteins exert a major regulatory role in the inflammatory and apoptotic cascade observed following brain ischemia, and might represent targets for neuroprotectants (30). Apoptosis is triggered by a cell death program at the mitochondrial level, resulting in cytochrome C release and ATP production when oxygen is available. This results in turn in caspase activation and cell death. Apoptosis is tightly regulated by pro- (bax, bad) and anti- (bcl2) apoptotic factors. Tyrosine phosphorylation stimulated by growth factors (NGF, BDNF) plays a major role in the inhibition of neuronal apoptosis. Anesthetics interfere with most of the steps of this process leading to programmed cell death. Activation of GABA-A receptors and reduction of excitotoxic glutamate release contribute to the neuroprotective effects of isoflurane. Sevoflurane attenuates the energetic consequences induced by ischemia in brain tissue (31). Cellular mechanisms such as activation of phosphorylation cascades of enzymes

playing a role in cell signaling and survival (MAP kinases, tyrosine kinases) may significantly contribute to the neuroprotective and antiapoptotic effects of volatile anesthetics. Isoflurane increases the survival of cortical neurons by stimulating calcium-dependent cell signaling, modulating MAP kinase ERK1&2 activities and increasing the expression of antiapoptotic and hypoxia-induced factors (32, 32). However, the neuroprotective effect of anesthetics remains ephemeral so far. Many anesthetics activate protein kinase C, possibly by binding to the regulatory domain of the enzyme at the diacylglycerol binding site (34). Protein kinase C stimulates both ERK1 & 2 and FAK phosphorylation. A short exposure (5 min) of all anesthetics except ketamine stimulates FAK phosphorylation via a PKC-dependent mechanism (26).

Anesthetic preconditioning consists in protection of the cerebral tissue against ischemic injury by a previous exposure of an anesthetic before the ischemic insult is applied. This is a general phenomenon that has been extensively reported in other organs, and primarily the myocardium. The plasmalemmal ATP-dependent K channels play a major role in the anesthetic preconditioning of myocardial tissue. The signaling pathway also involves activation P38 and ERK1-2 MAP kinases (35). These considerations also apply to the CNS. Sevoflurane preconditions brain tissue in a model of oxygen glucose deprivation, and this phenomenon is blocked by tyrosine kinase inhibitors (36). Dexmedetomidine also exerts a preconditioning effect which is likely to be mediated by both alpha2-A receptor- and imidazoline-II receptor-mediated actions (37, 38). Xenon also preconditions brain tissue via the MAP kinase, CREB and BDNF pathways (5, 35).

3.3. Neurotoxicity and cognitive dysfunction

In addition to their experimentally reported neuroprotective effects, anesthetics may also exhibit neurotoxic properties. NMDA receptor antagonists exhibit potential neurotoxicity in the early phase of the developing brain (39). Recent data support that isoflurane exerts a pro-apoptotic activity by stimulating protein expression of the beta-amyloid material, which represents a neurobiological substrate of Alzheimer's disease (40, 41). Hypothermia induced by anesthesia is responsible for Tau protein phosphorylation as well, and this effect can be prevented by maintaining normothermia (42, 43). The clinical relevance of these experimental findings remains to be determined. Particularly,

there is a need for a better understanding what is initiated by anesthesia per se, and what is relevant to the surgical inflammatory process in the generation of a postoperative cognitive decline frequently observed in elderly surgical patients (44).

In conclusion, the view of pharmacodynamic behavior of anesthetics in the brain has profoundly changed during the last decade. Anesthetics exert multiple effects on a restricted number of molecular targets in the CNS. Some of them may account for the hypnotic, immobilizing and amnesic effects of anesthetics. However, there is growing evidence that anesthetics also have the potential to initiate long term effects in the CNS, some of them being possibly involved in postoperative cognitive dysfunction. To better understand these changes related to anesthetic effects in the brain represents an exciting challenge for the next years.

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