Advances in the molecular basis of anaesthesiology

INTRODUCTION

The topic may be approached by defining the concept of anaesthesia, a term introduced by Oliver Wendell Holmes for defining the elimination of surgical pain, characterised by immobility, eliminating pain, amnesia and lack of consciousness, effects provoked by using anaesthetic agents reversibly affecting different areas of the central nervous system (CNS). These conditions were successfully met for the first time on the 16th of October 1846 in the Massachusetts Hospital when Dr. William Morton[1] administered ether by inhalatory route to a patient for the removal of a mass from the neck, a landmark for modern medicine, especially in surgical areas. New anaesthetics have been developed since then and attempts made to understand their mechanism of action (MOA) for ascertaining the components of anaesthetics.

Some investigators have tried to describe anaesthetics' MOA, such as Claude Bernard who described how the effect of anaesthetics could be caused via a common target for action in the CNS. Around 1900 Meyer and Overton, independently and then together, described the power of anaesthetics according to their solubility in lipids, establishing the Meyer-Overton hypothesis indicating cellular lipid membranes as being the main target for anaesthetic action.[2]

In spite of anaesthesiology having been practised for a broad diversity of surgical procedures from this moment on, it has only been since three decades ago that the mechanisms by which these drugs act and are reverted have been discovered due to scientific advances in molecular biology.[2-3]

Molecular biology, which focuses on studying life at molecular level, integrates several sciences such as genetics and genetic engineering for understanding different cellular systems' interactions including DNA–RNA relationships, proteins, metabolism and how all these interactions are related for the functioning of the cell.[4-6]

Important developments in molecular biology, such as genetic mapping, point mutations and developing knockout and knock-in mice (in which a gene is blocked or activated for understanding determined proteins' function in their absence), have led to understanding molecular biology's vertiginous advances.[4]

ANAESTHETICS' PHYSIOLOGICAL MECHANISMS

Understanding anaesthetics' MOA is made simpler when it is studied by describing how anaesthetics act on each of their components (immobility, hypnosis, lack of consciousness, amnesia).[5]

Immobility

Eliminating movement as a response to pain is primarily mediated at spinal cord level. At brain level anaesthetics selectively inhibit many sites, including the thalamus, reticular substance, cortical regions (motor and sensory) and subcortical regions. However, there is no definitive evidence about specific regions which are the target in the CNS for inhaled anaesthetics.[3]

Research using knockout mice has led to gamma-aminobutyric acid (GABA)A α2 receptors being related to relaxation related to diazepam[101] and the GABAA β3 subunit being related to immobility caused by propofol, etomidate and halothane.[102] No relationship with a single receptor has been found for inhaled anaesthetics since several interaction sites have been demonstrated. A 20% increase in minimal alveolar concentration (MAC) for isoflorane and sevoflorane can be observed when blocking GABAA β3 receptors; when glycine receptors are blocked there is a 20% increase in MAC; a 30% increase is obtained by blocking NMDA receptors and potassium channel TREK-1. The foregoing observations lead to it being thought that immobility probably cannot be related to a single type of receptor.[3-4-5-9-15]

Sedation and hypnosis

Even though sedation has been mostly defined as a decreased level of consciousness, reduced motor activity, altered speech, hypnosis and lack of response to verbal stimuli, correlation at molecular level is similar. However, a sedative effect has been implicated with cortical structures and hypnosis with the thalamus at neuroanatomical level. Sedation and hypnosis have been related to the inhibition of GABA receptors, mainly β2 and β3. Research has been fundamentally centred on propofol and eto-

midate with their action on these receptors mainly being located in the neocortex and the thalamus. Even though different studies with electroencephalography and positron emission images under the effect of sedation and hypnosis have demonstrated an overall decrease in cerebral consumption of glucose, this has mainly been observed in the aforementioned regions.[33-34] Benzodiazepine action is related to GABA receptor α1, α2, α3, α5 and β2 subunits, having different affinities according to the benzondiazepine used, thereby bestowing different powers; this has led to new benzodiazepines (i.e. bretazenil and imidazenil) being developed according to their affinity for subunits.[7-8]

Barbiturates cause sedation and hypnosis by acting on the GABA β3 receptor; at low concentrations this improves receptor functioning whilst increasing the concentration favours chloride entry even more without the need for binding to the receptor's subunit, favouring so-called "barbituric coma".[35-14]

Amnesia - memory

It is known that the reticular activating system, the thalamus, the bridge, the tonsils and the hippocampus also have areas which are involved in memory, learning, cognition, sleep and attention. However, even though sleep and anaesthesia are clearly different states, the networking of the subcortical neurons involved in sleep could become affected by anaesthetics. The tuberomamillar nucleus of the hypothalamus, modulated by GABA (especially by interactions between it and its receptor's α5 subunit), is related to states of sleep produced by intravenous and inhaled agents' sedative action. Anaesthesia-induced lack of consciousness is consistently associated with uncoupling the brain's posterior-anterior and interhemispherical electrical connection. In clinical practice this state is associated with the suspension of counting by the patient during anaesthetic induction.[2-3]

ANAESTHETICS' MOLECULAR MECHANISMS

Lipid action

It is known that general anaesthetics interact with lipid membranes, these being fundamental components of all cells according to Meyer and Overton; this theory has been used for explaining anaesthetic action at neuron level. Such rule relates to anaesthetics' liposolubility, in the sense that an anaesthetic agent becomes dissolved in the membranes thereby altering neuronal functions.

However, recent studies have demonstrated that increased temperature and substances such as *n*-alcohols and *n*-alkanes provoke the same changes in the membrane without producing anaesthesia. An important role has been found for protein receptors due to developments in a molecular biology.[13-14]

Protein action

Ion channels are proteins which regulate the flow of ions through the cytoplasmic membrane, being a variety of channels modulating a cell's electrical activity. They are related to anaesthetics' physiological action, such channels being sensitive to several inhaled and intravenous anaesthetics.[6-13]

Ion channels are sensitive to volatile and intravenous anaesthetics at an effective clinical concentration including the two "cysteine-loop" super families containing nicotinic acetylcholine receptors, serotonin type 3, GABAA and glutamate glycine receptors which are activated by N-methyl-D-aspartate (NMDA) or alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). Other channels involve potassium, especially TREK-1.[14-22-24]

GABA receptor

Gamma-aminobutyric acid (GABA) is the main cerebral inhibitory neurotransmitter; each receptor is a very complex heteromeric transmembrane protein which opens a permeable pore to Cl in response to the GABA ligand.[2] Rapid inhibition inotropic $GABA_A$ $GABA_C$ receptors and slow inhibition metabotropic $GABA_B$ Ca++permeable receptors have been recognised. The receptor is mapped on gene 1 of chromosome Gp21.3 in the HLA class I region, close to the HLA-F[5] gene; however, there is genetic expression for each receptor subtype.[16-17]

GABA synthesis, at the presynaptic site, begins from glutamate which is carboxylated by glutamic acid carboxylase. GABA, in turn, is degraded by GABA-transaminase (GABA-T) converting it to succinic semialdehyde which is metabolised by succinyl semialdehyde dehydrogenase to succinic acid. GABA is stored in the presynaptic vesicles and released to the exterior like other neurotransmitters by calcium-modulated action, where cytosol can also be captured by a transporter. On the postsynaptic side, GABA presents a pentameter structure consisting of five subunits of alpha, beta and gamma varieties

where receptor activator sites are found, provoking chloride influx via the ions channels, hyperpolarising the cell and impeding excitability.[6]

Evidence has been presented that there are different sites in the GABA receptor subunits specifically recognising medicaments such as barbiturates, agonist (midazolam, diazepam) and antagonist (flumazenyl) benzodiazepines (mainly subunit α) from propofol, mainly acting at subunit β, β2 and β3 level, respectively. They are used in experimental work with laboratory animals. All these substances can modulate GABA action.

Clinical practice has shown that the CNS is the target organ for benzodiazepines, barbiturates and inhaled anaesthetics. However, it should be stressed that these drugs were developed before the diversity of existing GABA receptor subtypes became understood. New research will surely lead to a better understanding of the roles which each GABA$_A$ receptor subunit plays regarding the action of anaesthetics.[16-18-19-20]

GABA receptor formation

The GABA receptor has five different subunits: alpha, beta, gamma, delta and epsilon; the alpha subunit has 6 isoforms, beta 4, gamma 3, delta 1 and epsilon 2.

Five subunits must be combined to form chlorine channels; each subunit's composition changes affinity for additional allosteric sites and the efficacy of the GABA agonist site. There are four transmembrane domains for each subunit (M1, M2, M3 and M4) having hydrophobic properties leading to their inclusion in the lipid bilayer. Nineteen combinations have been found in the CNS to date, the most frequently occurring ones being α1β2γ2 (60% of GABAα receptors), α1β3γ1 (15%) and α3βnγ2 (10 – 15%).[19-20]

Clinical experimentation with GABAa

Reduced immobilisation response for the δ subunit in knockout mice has only been demonstrated with pregnenolone but not for anaesthetics such as phenobarbital or etomidate, thereby demonstrating that this subunit plays a more extrasynaptic role. Blocking the β3 subunit caused a drop in the level of hypnosis induced by etomidate and diazepam and partly in immobility caused by halothane, enfluorane and etomidate.

Experiments with point mutations have also demonstrated important effects. A 50% decrease in the effect of etomidate and propofol has been demonstrated by changing the position of a methionine for an asparagine in the β subunit chain.[18-21]

Changes in subunit α have also been demonstrated to have had many effects: the α1 subunit with anterograde amnesia and α2 with anxiolysis; myorelaxant action is related to subunits α2 and α3.

It has been demonstrated that blocking β2 and β3 for inhaled anaesthetics could cause a 30% increase in MAC, thereby causing immobility with halothane and enfluorane.[21]

OTHER ION CHANNELS [2]

Glycine receptors

Glycine receptors are mainly chlorine-mediated inhibition receptors which are located throughout the CNS, but mainly in the spinal medulla. They are formed by a pentameric channel: 3 α subunits having 4 subtypes and 2 β subunits having 3 subunits (few studies are available).

Their relationship with anaesthetics has been described, mainly with 20%-55% inhaled anaesthetic-mediated immobility for isoflorane and 20% for halothane, MAC becoming increased when they are blocked.[25-3]

Glutamate receptors

Glutamate is the main CNS exciter neurotransmitter, exercising its action via metabotropic and inotropic receptors. It was described as a neurotransmitter more 40 years ago, being related to multiple neuronal functions. There are also pre- and post-synaptic receptors, pre-synaptic receptors mainly being metabotropic and post-synaptic ones inotropic.

There are 3 types of inotropic receptor: NMDA, AMPA and kainate. They are so described due to their exogenous ligands, each one offering different types of permeability for Na and Ca.

The large diversity of glutamate receptors in the CNS has led to difficulty in establishing their relationship with anaesthetics. Studies have shown how nitrous oxide, xenon and ketamine are potent glutamate receptors antagonists (especially AMPA receptors). NMDA receptors are especially antagonised by nitric oxide and experimental studies have shown how isoflorane interacts with GLuR6 kainate receptor subunits inhibited at medullar level, thereby causing increased MAC. AMPA receptors and different subunits have been related to xenon's ability to induce immobility.[3-4-26]

Serotonin receptors

Serotonin is a neurotransmitter which is secreted by many cells including neurons, chromafine cells

Advances in the molecular basis of anaesthesiology - Madiedo CN., Perea DJ.

from the gastrointestinal system and platelets. They are related to many functions, including neurotransmission in the enteric system and inhibiting many stimuli in the CNS related to controlling emotions, sleep and temperature in several CNS nuclei.

The main member of the serotonin receptor family is the serotonin 5-HT3subtype which can be affected in different ways since it can be boosted by halogenates or can be inhibited by barbiturates or ketamine. Studies on with experimental animals which have been given specific serotonin capture inhibitors in determined cerebral nuclei have reported an increase in MAC necessary for causing immobility. Serotonin receptors are related to an altered state of consciousness with barbiturates.[3-27]

Voltage-dependent potassium channels

Amongst ion channels, those for potassium have the greatest phylogenetic diversity. Potassium flow via these pore-forming proteins plays a relevant role in cell physiology; for example, the high permeability at rest to this ion produces the potential for action at rest and contributes towards regulating cellular volume in this state. There are 3 large families of potassium channels according to their amino acid sequence homology:

a- Family of 1 pore and 6 transmembrane segments;

b- One pore and 2 transmembrane segments; and

c- Two pores and 8 segments, the TREK and TASK channels, strongly implicated in the action of anaesthetics, belonging to the last family. TREK-1 channels are related to the immobility caused by inhaled anaesthetics at medullar level. TASK channels related to base ganglions are related to immobility caused by propofol.[28-29]

Nicotinic acetylcholine receptors

Nicotinic receptors are cholinergic ion channels (i.e. they are able to respond to a chemical mediator such as acetylcholine. They are so called because they can be activated by nicotine, unlike receptors which are activated by muscarine.

Nicotinic receptor studies in anaesthesiology have been related to nearly all anaesthetics (both inhaled and intravenous ones) causing their inhibition.

Studies carried out on the *Xenofus oocites* model (an Asiatic frog having a predominance of nicotinic acetylcholine receptors (nAChR)), using different anaesthetics, showed that the main inhibitor of these channels was the standard one. Ketamine competitively inhibited channel opening at ganglion level (predominantly on α4β2 receptors); the α4β4 subunit has been the substrate for action in the *Xenofus oocites* model for nitrous oxide, propofol and xenon. The MOA for etomidate and thiopental is little known since no major effect on acetylcholine receptors has been found to date.[5-31-32]

Classifying anaesthetics according to action site

Anaesthetics' MOA on different receptors in the CNS has meant that anaesthetics can be classified according to their site de action, thereby correlating their essential clinical characteristics and their molecular target.

CONCLUSION

Research carried out from the time of the Meyer-Overton unitary theory up to genetic studies and advances in molecular biology have all been important processes in describing and understanding the complex mechanisms occurring at molecular level in the CNS due to inhaled or intravenous anaesthetic drugs being used. Physiologically, the actions are very different and compromise different regions of the CNS: immobility at spinal medulla level, amnesia and lack of consciousness at cerebral level and autonomic responses in specific areas of the bulb and protuberance. All these actions are very specifically modulated to different degrees by different receptors (with their different subtypes) regarding the response caused by anaesthetics.[3-5]

Based on the foregoing, studying and being aware of the molecular basis of anaesthesia is of vital importance in understanding the physiological phenomena produced by anaesthetics and their applicability in clinical practice. The drugs affecting the CNS will be designed more rationally in the near future, having more specific and effective action and having fewer collateral effects.[2] Remaining ignorant of molecular biology will make it impossible to take advantage of new discoveries in anaesthetic pharmacology for using them in anaesthetic practice in the future.

Anaesthesiologists recognising the importance of these molecular and biological implications of anaesthetics will have at their disposal knowledge of a wide range of medicaments to enable a more critical selection of more reliable drugs for each patient, thereby contributing towards making practice in anaesthesiology safer and more fascinating.

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Advances in the molecular basis of anaesthesiology - Madiedo CN., Perea DJ.