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Anaesthetic mechanisms: update on the challenge of unravelling the mystery of anaesthesia

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Abstract

General anaesthesia is administered each day to thousands of patients worldwide. Although more than 160 years have passed since the first successful public demonstration of anaesthesia, a detailed understanding of the anaesthetic mechanism of action of these drugs is still lacking. An important early observation was the Meyer-Overton correlation, which associated the potency of an anaesthetic with its lipid solubility. This work focuses attention on the lipid membrane as a likely location for anaesthetic action. With the advent of cellular electrophysiology and molecular biology techniques, tools to dissect the components of the lipid membrane have led, in recent years, to the widespread acceptance of proteins, namely receptors and ion channels, as more likely targets for the anaesthetic effect. Yet these accumulated data have not produced a comprehensive explanation for how these drugs produce CNS depression. In this review, we follow the story of anaesthesia mechanisms research from its historical roots to the intensely neurophysiologic inquiries regarding it today. We will also describe recent findings that identify specific neuroanatomical locations mediating the actions of some anaesthetic agents.

Keywords

anaesthetic mechanisms; anaesthetic targets; anaesthetics; ion channels; receptors

Introduction

`*Healthy discontent is a prelude to progress*' – Mahatma Gandhi

Over time humankind has employed an array of natural medicines and physical methods to alleviate pain and suffering. Ancient Indian and Chinese texts record the beneficial analgesic effects of cannabis and henbane. In Egypt around 3000 B.C., the opium poppy, hellebore, beer, and the legendary mandrake were used for similar purposes [1]. Other approaches to deal with surgical trauma and pain relied on physical methods such as cold, nerve compression, carotid artery occlusion or infliction of a cerebral concussion. The effectiveness of these historical agents is unknown but allude to an ever present need.

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A new era of anaesthesia arose as one of the advancements of the Enlightenment. It began with the isolation of oxygen and the synthesis of nitrous oxide in the 1770's by Joseph Priestley. Initially, nitrous oxide was used solely as an intoxicant. American dentist, Horace Wells was the first to recognize the anesthetizing potential of nitrous oxide; however, his attempt to produce surgical anaesthesia in 1845 with this weak anaesthetic was a miserable failure.

Another compound, known as `sweet vitriol' by Paracelsus and renamed ether in 1730 by German chemist Frobenius proved to be more potent. It was used for the first successful public demonstration of surgical anaesthesia, conducted by William Thomas Green Morton in Boston in 1846, the year after Wells' failure [1]. Prior to this, Crawford Long, an American physician practicing in Georgia had performed surgery under ether anaesthesia as early as 1842. In 1847, Scottish physician Sir James Young Simpson succeeded in demonstrating anaesthesia with chloroform, which had been discovered in 1831 by Souberain, Guthrie and Liebig. Chloroform was used by John Snow to oversee the painless delivery of Queen Victoria's eighth child, Leopold, in 1850 [1]. Fast forward to today, a large number of drugs are available to the modern anaesthetist to produce general anaesthesia, from intravenously administered hypnotics to halogenated vapours and gaseous agents such as xenon. The intravenous hypnotics, which provide a smooth induction of anaesthesia, include barbiturates such as sodium pentothal, introduced in 1934 in America by Lundy; etomidate, introduced and studied by Doenicke in 1973, and propofol, which has been widely accepted since its introduction in 1977. The structures of these disparate agents and others are shown in Fig. 1. This review will take a broad look at the mechanisms of action of both inhalational and injectable anaesthetics and attempt to discriminate where they may overlap and where they may diverge.

Research efforts have pursued a reductionist path to approach the difficult problem of assigning the clinical effect of an anaesthetic to action at a single biological site. This so-called `unitary theory' has animated efforts to produce a single molecular definition of the site of anaesthesic action. Subsequent research has revealed a multitude of targets and produced a more complex picture of how clinically relevant doses of anaesthetics affect molecular targets throughout the central nervous system (CNS). Ironically, in recent years research groups have discovered that a few discrete sites within the CNS may indeed mediate the action of some general anaesthetics.

What does anaesthesia represent and where does it work?

> `*I believe in a long, prolonged, derangement of the senses in order to obtain the unknown*.' – Jim Morrison, The Doors

Anaesthesia embraces three controlling, yet reversible characteristics: immobility, amnesia and unconsciousness [2–4]. A more comprehensive definition of anaesthesia could include control of pain (analgesia), muscle relaxation, suppression of reflexes, prevention of nausea and vomiting and even reduction of long-term effects such as postoperative cognitive dysfunction [5]. A wide definition such as this encompasses many areas of clinical concern for anaesthetists and highlights the failure of any single drug to act effectively on all of these components.

Immobility

Immobility is the easiest anaesthetic endpoint to measure. In 1965, Eger and colleagues introduced the MAC (minimal alveolar concentration) concept, which quantifies the potency of an inhalational anaesthetic [6]. They defined 1.0 MAC as the partial pressure of an inhalational anaesthetic in the lungs, at which 50% of a population of nonrelaxed patients remained immobile at the time of a skin incision [7].

At the physiological level, it is clear that surgical immobility is based on the interaction between a volatile anaesthetic and the spinal cord. Several studies in animal models over the past decade provide evidence for the direct effects of anaesthetics on subcortical structures to prevent motor responses to painful stimuli [8–11]. The measurement of MAC is now widely accepted to be a spinal level phenomenon.

Recently, Eger *et al*. proposed a new view of MAC [12]. They compiled powerful arguments against the unitary model by summarizing data from their lab [13] and others to conclude that only a few of the ion channel targets that have been proposed as putative sites of anaesthetic action remain viable candidates to explain MAC. Instead they suggest that immobility may result from nonspecific actions within the spinal cord. A major conundrum emerging from these studies is to understand how it is that the behavioural reflex (movement) in response to the nociceptive signalling is completely attenuated while nociceptive neurotransmission to the spinal cord persists at surgical planes of anaesthesia.

Amnesia

The effect on memory arises from several locations in the CNS including the hippocampus, amygdala, prefrontal cortex, as well as regions of the sensory and motor cortices [14]. Much work has gone into understanding how anaesthetics influence one or more of these centres to produce differential effects on type of memory. These studies differentiate explicit memory, i.e. specific awareness or consciousness under anaesthesia from implicit memory, the unconscious acquisition of information under adequate levels of anaesthesia. These studies have generally found that the formation of both forms of memory are prevented at low MAC values (0.2–0.4 MAC) [15]. However, there may be a greater possibility of implicit memory at these levels of anaesthesia [16–19]. Prevention of explicit memory (awareness) has spurred the development of monitors such as the bispectral index (BIS), electroencephalogram (EEG) and entropy monitoring or auditory evoked potential recording to recognize inadequate planes of anaesthesia. The importance of implicit memory during anaesthesia remains an unresolved question.

Consciousness

Probably the biggest and most difficult question in neuroscience research involves identifying the physiological processes and anatomical locations responsible for the formation of human consciousness. The ability of anaesthetic drugs to influence this peculiarly human characteristic is even less well understood. Currently, leading neuroscientists studying consciousness acknowledge three regions of the CNS that appear to participate in generating our personal awareness: the cerebral cortex, the thalamus and the reticular activating system [20–24]. These three regions are thought to interact as a cortical system via identified impulse pathways, in a distributive way to make us awake, aware and perceiving [25,26].

This conceptualization does not help to narrow down the site of consciousness since a cortical system encompasses the whole cerebral cortex plus other closely associated regions such as thalamus, cerebellum and basal ganglia [25]. Before his death, Francis Crick, and collaborator Christof Koch proposed special significance for the claustrum, a thin layer of neurons with extensive reciprocal connectivity to cerebral cortex, as an important brain structure for consciousness [27]. Few electrophysiologic investigations of this brain region have been conducted, and none have focused on determining the effects of anaesthetics.

The neuronal correlates of consciousness (NCC) were also defined by Crick and Koch as the minimal neuronal mechanisms sufficient to support a specific conscious perception [25]. Two competing theories focus on different anatomical units as the basis of NCC. One hypothesis, put forth by Koch attributes each purposeful experience to a unique activation pattern within

a group of neurons [28]. In contrast, Greenfield attributes each such experience to the synchronized activation of neurons over the entire cortex in a sphere of coordinated combination, which is deactivated upon the arrival of a new stimulus [28]. Koch's theory is neuron-specific whereas Greenfield's is signal-specific. Depending on which, if either of these ideas are proven true, anaesthetics may disrupt consciousness by disrupting the interaction either of a small cadre of neurons or of global brain signalling patterns.

Monitoring consciousness

These descriptive observations can not bring us closer to knowing how anaesthetics induce unconsciousness until a more complete understanding of consciousness is reached. Nevertheless, a gradient of clinical disruption of CNS function by volatile anaesthetics may be described. As anaesthetic is introduced, the three basic elements of the anaesthetized state, amnesia, unconsciousness and immobility, are sequentially impaired. At low concentrations (0.1–0.3 MAC), anaesthetics produce sensory distortions and fragmentation, sleepiness and memory loss as individuals become increasingly more difficult to arouse. Sedation such as this, with low dose volatile agents, has been found to act primarily on the cortex itself [29]. At MAC-awake, generally around 0.3–0.5 MAC, the response to verbal command is lost in 50% of patients, and they are on the verge of unconsciousness. Blunting consciousness at this stage by some intravenous anaesthetics has been localized to a small subcortical brain stem region called the tuberomammillary nucleus [30]. Finally, immobility to noxious stimulus is achieved at or above 1.0 MAC.

Many anaesthetists have adopted BIS to monitor cerebral function and depth of anaesthesia. Awareness under anaesthesia represents a failure of our agents or techniques to sufficiently blunt consciousness. Avidan *et al*. recently added more evidence to the growing consensus that this technique does not diminish the incidence of awareness during anaesthesia [31]. BIS may be influenced by the degree of muscle relaxation [32], and although BIS can reflect disruption of the cerebral cortex [33], the choice of anaesthetic itself can influence BIS measurements [34]. In addition, no differences could be detected for key memory centres such as the hippocampus and various neural circuits, which exhibit different anaesthetic sensitivity [35].

Holistic versus specific regional effects of anaesthetics

How then might anaesthetic agents have their effect on consciousness? Do they specifically target Koch's limited groups of neurons; do they exert broad-based effects on specific signalling processes within neural networks [36]? As with our uncertain understanding of the basis of NCC, our ability to identify disruption of neural signalling caused by anaesthetics lacks sufficient resolution. The effects on brain activity of the inhaled anaesthetics halothane and isoflurane, and the intravenous anaesthetic propofol have been studied by assessing regional glucose metabolism using PET scanning [37–39]. The inhaled agents produced a global decrease in brain glucose metabolism at the point at which consciousness is lost. Propofol produced a slightly different pattern with a significantly lower decrease in glucose metabolism for subcortical regions than in cortex. Fiset and colleagues were able to confirm the ability of propofol to reduce metabolism in the medial thalamus and other important `arousal' regions [40].

These findings have been integrated into a hypothesis that anaesthetics interfere with thalamocortical oscillations, which appear to be relevant for consciousness [41] or for corticothalamic reentry [42]. It is known that anaesthetics can act to hyperpolarize thalamocortical neurons and alter them from a tonically active state to a `burst-firing' mode. This change may then convert cortical activity from rapidly oscillating field potentials in the range of γ frequencies to the slower δ and θ oscillations [36,41]. Interestingly, the differential partitioning of anaesthetics into the lipids comprising the grey and white matters of the brain

may be an important factor at these sites. White matter has a higher tissue-gas partial pressure compared to grey matter, and values for the thalamus, hypothalamus and hippocampus lie in between [43].

At the cellular level, the influence of anaesthetics may act either by strengthening inhibition or by diminishing excitation within various parts of the brain. However, it is clear that not all neurons that may be involved in the formation of consciousness are constrained under the influence of anaesthetics [36]. This is the same problem identified by Eger *et al.* in that neural transmission from the periphery to central pain centres still persists even at supra-MAC levels of anaesthesia [12]. Nevertheless, our current state of understanding supports the following framework: sensory stimuli conducted through the reticular formation of the brain stem into supratentorial signalling loops, connecting the thalamus with various regions of the cortex, are the foundation of consciousness and that these neural pathways involved in the development of consciousness are disturbed by anaesthetics [44].

The lipid bilayer as the site – the Meyer-Overton Correlation

> `*No diet will remove all the fat from your body because the brain is entirely fat. Without a brain, you might look good, but all you could do is run for public office*.' – George Bernard Shaw

At the turn of the 19th Century, work carried out by botanist Charles Ernest Overton [45,46] in Zurich and pharmacologist Hans Horst Meyer [47] in Marburg independently identified a strong correlation between the potency of an anaesthetic and its solubility in olive oil (Fig. 2). This meaningful correlation, which held true for over a 1000-fold range of anaesthetic solubilities, suggested a common unitary mechanism. Their recognition of the importance of lipid solubility was the first attempt to explain the functional mechanism of anaesthetics.

Since the recognition of the Meyer-Overton correlation, several lipid-based theories evolved over the last century to explain anaesthetic phenomenon. These ideas included anaesthetic-induced volume expansion of the cell membrane [48], increased fluidity of the cell membrane [49] and increased lateral surface pressure [50,51]. Another impetus for these hypotheses stemmed from the dramatic findings that animals under conditions of increased hydrostatic or barometric pressure were resistant to the effects of volatile anaesthetics [52,53]. These observations, termed pressure reversal of anaesthesia, led to the further development of theories focused on the disruption of membrane structure by increased membrane fluidity as an explanation for the action of anaesthetics. [54–58] These theories were attractive because they relied on purely thermodynamic considerations and could account for the wide range of chemical structures represented by anaesthetic molecules. However, these ideas conflicted with other whole organism data showing that elevated body temperature, which disrupts membrane fluidity as much as anaesthetics, actually increases MAC, not reducing it as these hypotheses would predict [59].

Eclipse of Meyer-Overton

Over the last 20 years, additional doubts have arisen about the focus on the lipid membrane as the site of anaesthetic action that arose from the Meyer-Overton correlation.

Luciferase

Fireflies employ the soluble enzyme luciferase to generate flashes of light. When the enzyme was purified lipid-free and studied in the presence of various anaesthetics, its bioluminescent function was impaired according to the potency predicted by the Meyer-Overton correlation

[60]. These data demonstrated that proteins may also be affected by anaesthetics according to the Meyer-Overton correlation.

Nonimmobilizers

Eger *et al*. identified a large number of compounds whose structure and lipid solubility would suggest general anaesthetic ability but when tested on rats at concentrations predicted by the Meyer-Overton correlation did not induce anaesthesia or induced it at higher than expected concentrations [61,62]. Thus, these compounds fell off the Meyer-Overton correlation line and were labelled nonanaesthetics. More precisely, these compounds (e.g. dichloro-hexafluorocyclobutane) have come to be termed nonimmobilizers because they do not prevent movement in response to noxious stimuli in the rodent MAC assay but are able to inhibit the higher CNS functions of learning and memory at concentrations near the predicted Meyer-Overton effective concentration [63,64]. They have, thus, come to be important control compounds for studies seeking to identify a specific mechanistic target of anaesthetics. As such, a legitimate site of action of anaesthetics for MAC ought to be unaffected by nonimmobilizer compounds.

Cut-off effect

The `cut-off' effect describes a relationship between molecule size and anaesthetic potency. Within a series of chemically homologous anaesthetics such as the n-alkanes, potency increases with increasing molecular size, as predicted by the Meyer-Overton correlation, until a critical molecular dimension is reached (cut-off). Above this size, members of the series lose their anaesthetic effect despite increasing lipid solubility [65]. These results can be interpreted to mean that the site of action for anaesthetics represents a cavity or binding pocket of restricted size that constrains the effective size of drugs that induce anaesthesia. The fact that lipophilic drugs above this cut-off size fail to induce anaesthesia demonstrates another violation of Meyer-Overton.

Stereospecificity

A stereoselective difference in potency of anaesthetics has been shown for intravenous and inhalational anaesthetics. With stereoisomers of isoflurane, Lysko *et al*. found that the (S+) enantiomer was 50% more potent than the (R−) enantiomer in rats using standard MAC assay [66]. This result was confirmed by the Franks and Lieb group who determined a 40% increased potency for loss of righting reflex for (S+) over (R−) isoflurane when injected intravenously [67]. Because stereoselectivity is presumed to arise from the interaction of the drug with an optically active site on the protein, these studies provide evidence for a specific molecular interaction rather than a more generalized physico-chemical effect of the anaesthetics. The criteria of stereoselective potency differences has, thus, become an important discriminator for a legitimate site of anaesthetic action.

The studies described above led the anaesthesia mechanism research community away from purely lipid-based explanations for the action of anaesthetics. The new focus came to rest on protein components of the membrane as potential targets for general anaesthetics. The realization that proteins also contain lipophilic domains supports the continuing importance of the Meyer-Overton correlation [68]. And while it remained a formal possibility that anaesthetics interact with a single protein site, the sudden increased number of protein candidates has come to make the unitary hypothesis of anaesthesia less and less tenable [69, 70].

Protein targets for volatile anaesthetics

> `*The great thing in the world is not so much where we stand, as in what direction we are moving*.' – Oliver Wendell Holmes, American jurist

The studies by Franks and Lieb with luciferase [60] stimulated interest in a whole new repertoire of protein targets for anaesthetics. At that time, improvements in the electrophysiological analysis of ion channel activity and in molecular cloning techniques were enabling researchers to study the molecular pharmacology of anaesthetic drugs on pure populations of membrane receptors and ion channels. Other research groups demonstrated that soluble cytoplasmic proteins, such as protein kinase C, an important signal transduction enzyme involved in regulating the release of neurotransmitters and ion channel activity [71, 72], and mitochondrial proteins are also affected by anaesthetics and should not be overlooked [73]. Even structural elements in support cells of the CNS such as gap junctions of astrocytes, which could influence communication between astrocytes, were found to be susceptible to the effects of anaesthetics [74]. The problem for investigators shifted from discovering the key physicochemical effect of anaesthetics on a homogenous lipid membrane to the new burden of too many possible protein targets.

Diversity of possible functional sites

We now know that the human genome contains the coding sequence for between 20 000 and 21 000 proteins [75]. In order to winnow through the abundance of potential clinically relevant functional sites for anaesthetics, the following criteria have been put forward [76]:

1. Anaesthetics must produce a reversible effect at a functional site with clinically relevant concentrations;
2. A functional site must be situated at a plausible anatomical location to mediate the specific behavioural effects of an anaesthetic;
3. Stereoselectivity of anaesthetic effects in vivo should duplicate the stereoselective effects observed in vitro;
4. A functional site should be insensitive to the effects of nonimmobilizers.

These requirements are met by several different receptors and ion channel families, which now constitute the leading molecular candidates for mediating the actions of anaesthetics (Table 1 and Table 2) [12,77]. Before considering specific proteins, we start with an exploration of some theoretical questions about the effect of anaesthetics on them.

Specific or nonspecific interactions with proteins

By virtue of their complex structure, proteins as a possible site of anaesthetic action are presumed to contain a specific binding site within their folded structure to mediate a functional interaction between protein and anaesthetic. But how can the variety of chemical structures (Fig. 1) capable of causing anaesthesia interact with such functional specificity? The best evidence at present is that anaesthetic molecules exert their effects on proteins by occupying gaps or pockets.

Proteins are known to harbour packing defects or cavities that may be critical for protein function [78]. Binding cavities for anaesthetics in functionally relevant proteins have been identified, and occupation of these domains by anaesthetic molecules can stabilize the protein in its native, folded state [79]. These interactions may occur with low affinity, consistent with the fact that clinical concentrations of general anaesthetics fall into the tens to hundreds micromolar range. So these postulated sites have a dual nature. They are specific in that they may exert an allosteric effect on the function of the protein but are nonetheless unspecific in

that they can accommodate a variety of molecular profiles within the folded structure of the protein [80]. Entry to these sequestered areas of a complex, folded protein structure will depend on a molecule's ability to penetrate hydrophobic amino acid sequences, thus, abiding by constraints of the Meyer-Overton correlation.

Presynaptic versus postsynaptic interaction

Anaesthetics may affect neurons at various functional levels, but the primary focus has been on the synapse. A presynaptic action may alter the release of neurotransmitters [81,82], while a postsynaptic effect may change the frequency or amplitude of impulses transversing the synapse [72,83,84]. From a functional standpoint, the action of anaesthetics may arise from changing the balance between inhibition and excitation of neural transmission within the CNS. Studies on isolated spinal cord tissue have demonstrated that excitatory transmission is impaired by anaesthetics much more strongly than inhibitory transmissions are potentiated to contribute to a mechanism of immobility [83,85]. To add to the complexity, the change in this balance could occur on the afferent side, sensing the intensity of the nociceptive stimulus, or on the efferent side, controlling movement [86]. Moreover, these two arms of the functional CNS may have varying sensitivity. Effects on sodium or potassium channels, which have a predominant role in axonal conduction as well as for determining the resting membrane potential, could mediate a similar effect [84,87].

Thus, chloride channels (gamma-aminobutyric acid $(GABA)_A$ and glycine receptors) and potassium channels (K_{2P}, possibly K_v and K_{ATP} channels) remain the primary inhibitory ion channels that fulfil the criteria for a legitimate anaesthetic action site [88–93]. On the excitatory side, anaesthetic-induced inhibition of ion channels activated by acetylcholine (nicotinic and muscarinic receptors) [94,95], excitatory amino acids (amino-3-hydroxy-5-methyl-4-isoxazol-propionic acid (AMPA), kainate and N-methyl-D-aspartate (NMDA) receptors) [96,97] and serotonin (5-HT2 and 5-HT3 receptors) are currently held as possible ion channel targets of anaesthetic action [87]. Below we consider in vitro and in vivo data on these channels.

Anaesthetic effects on inhibitory ion channels

$GABA_A$ and glycine receptors

Clinical concentrations of volatile anaesthetics are able to activate $GABA_A$ and glycine receptors expressed in vitro using heterologous expression systems as well as in vivo on the postsynaptic membrane [98,99]. The enhanced GABA-activated chloride current causes hyperpolarization of the neuronal membrane to reduce neuronal activity (Fig. 3a). In contrast, the gaseous anaesthetics xenon, nitrous oxide and cyclopropane as well as the intravenous agent ketamine have minimal or no effect on $GABA_A$ receptor subtypes [97,100–103]. Another population of $GABA_A$ receptors, the so-called extrajunctional $GABA_A$ receptors, can be activated by very low concentrations of GABA and are distinguished structurally from synaptic $GABA_A$ receptors. Currents passed by these receptors are potentiated by low concentrations of volatile anaesthetics and are widely expressed in important brain regions such as the hippocampus, thalamus, cortex and cerebellum [104]. The relevance of extrajunctional $GABA_A$ receptors in anaesthesia mechanisms is an open question.

The intravenous anaesthetics propofol and etomidate also enhance $GABA_A$ receptor function to produce immobility [105–107], and some molecular interaction sites have been elucidated. Rudolph *et al*. discovered a point mutation on the mouse α1 $GABA_A$ subunit that moderated whole animal benzodiazepine-induced behavioural patterns [108]. Barbiturates act on $GABA_A$ receptors depending on their concentration at the receptor by means of positive allosteric modulation, direct activation and inhibition. Drafts and colleagues identified a mutation in the $GABA_A$ α subunit that abolishes the action of barbiturates; however, activation

by GABA or potentiation by etomidate were not affected [109]. Alcohol also enhances $GABA_A$ mediated transmissions, which may play a role in mediating its intoxicating effects [110].

With the help of chimeric channel constructs, Mihic and colleagues identified a domain, 45 amino acids in length, relevant for mediating the effect of volatile anaesthetics and etomidate [111], but not propofol [88]. Further mutation of $GABA_A$ receptor subunits revealed two key amino acids involved in the interaction with volatile anaesthetics that are closely placed in the primary sequence. Hence, these residues may contribute to a binding pocket for the anaesthetic [112].

The role of inhibitory $GABA_A$ and glycine receptors has been well studied at the spinal level. To suppress a motor response, as determined by measurement of MAC, spinal injections of glycine receptor antagonists appear to have greater effect on MAC than $GABA_A$ receptor antagonists [87]. Possible spinal functional sites for inhalational anaesthetics include both glycine and glutamate receptors [12,113].

Potassium channels

Franks and Lieb identified an isoflurane-activated potassium current in specific neurons of the freshwater snail Lymnaea stagnalis [114]. This current had the characteristics of a leak or background K channel because it lacked voltage-dependent activation, was noninactivating and passed currents closely predicted by the Goldman-Hodgkin-Katz equation for ion conduction through a passive, K-selective pore [115]. Subsequently, a unique family of K subunits with two pore-lining sequences (K_{2P} channels) was discovered that had a wide phylogenetic range and was activated by volatile anaesthetics at clinically relevant concentrations [91,116,117]. Activation of these background K channels in response to volatile anaesthetics results in hyperpolarization and silencing of neuronal activity (Fig. 3) [114,118]. Members of the family can also be activated by xenon [119] and nitrous oxide [120], and differentially activated by isoflurane stereoisomers [121].

Evidence from K_{2P} knockout mice has further implicated these channels in the mechanism of action of volatile anaesthetics. TWIK-related K^+ channel-1 (TREK-1) knockout mice were resistant to the effects of six volatile anaesthetics as determined by the standard MAC assay [122]. Knockout mice in which other members of K_{2P} channel family have been inactivated (TWIK-related acid-sensitive K^+ channel-1 (TASK-1) and TASK-3) also show some resistance to the anaesthetizing action of volatile anaesthetics. Additional studies with multiple K_{2P} knockouts will be needed to understand their full importance.

Other potential K channel targets include voltage-gated K channels (K_v) and ATP-activated K channels (K_{ATP}). K_v channels were first isolated from mutant Drosophila that displayed an abnormal `shaking' reaction upon exposure to ether [123]. The Shaker phenotype arose from inactivation of a voltage-gated K channel gene, but in vitro studies of the effects of anaesthetics on this channel family has found them to be inhibited at supra-clinical anaesthetic concentrations [124,125]. Likewise, administration of K_{ATP} channel blocking drugs (pinacidil and cromakalim) into the neuroaxis did not change isoflurane MAC [126]. Thus, the primary focus of the anaesthetic mechanism involving K channels remains on background K channels.

Effect of anaesthetics on excitatory ion channels and neurotranmission

Glutamatergic ion channels

Figure 4 depicts a model of an excitatory synapse. Glutamatergic neurotransmission can occur via activation of three distinct families of ligand-gated ion channels: AMPA, kainate or NMDA receptors. The gaseous anaesthetics, xenon, nitrous oxide and cyclopropane, as well as the

intravenous agent ketamine have been shown to suppress excitatory, glutamate-mediated synaptic transmission by blocking NMDA receptors on the postsynaptic membrane [97,100–103]. In addition, enflurane and urethane inhibited NMDA-stimulated excitatory flow in NMDA-expressing Xenopus oocytes [127,128]. Blockade of AMPA receptors can decrease MAC by 60% [129], and inhaled anaesthetics potentiate currents through kainate receptors containing a GluR6 subunit [130]. Hollmann *et al*. were able to demonstrate a reversible dose-dependent inhibition of recombinant NMDA receptors by isoflurane, sevoflurane and desflurane [131]. These in vitro findings support a postsynaptic role of glutamate receptors in anaesthetic action. Volatile anaesthetics may also suppress the excitatory glutamatergic transmission via presynaptic inhibition of glutamate release [132]. However, studies with knockout mice have up to now failed to find a significant role in vivo [133–135].

The molecular site of action for xenon and isoflurane to inhibit NMDA receptors occurs by binding to the glycine coagonist site [136]. This finding may lead to the design of new anaesthetics, as some clinically well tolerated neuroprotective compounds are known to bind to this site as well.

Neuronal acetylcholine receptors

The primary role of nicotinic acetylcholine receptors in the CNS appears to be the modulation of synaptic conduction [137]. Many volatile anaesthetics inhibit acetylcholine receptors at clinical concentrations, but administration of nicotinic or muscarinic antagonists within the neuroaxis does not change anaesthetic potency [138,139]. Rather, these receptors appear to be more involved in modulating nociception than in mediating anaesthetic-induced immobility [140,141].

Adenosine receptors

Administration of adenosine or adenosine agonists can decrease MAC in vivo (rat and dog models) [142,143]. However, administration of a subtype-selective adenosine antagonist does not change halothane MAC, arguing against a significant role in inhaled anaesthetic mechanisms.

Serotonin receptors

The neurotransmitter serotonin causes neuronal signalling by activating a ligand-gated channel (5-HT3 subtype) as well as G-protein coupled receptor subtypes (5-HT2 and others) [144]. Inhaled anaesthetics block activation of 5-HT2A receptors and reduce nociception. However, the nonimmobilizer 1,2-dichlorohexafluorocyclobutane causes the same degree of inhibition of 5-HT2A receptors as halothane at a 1 MAC equivalency [145]. Blockade of 5-HT3 receptors by ondansetron does not alter MAC of isoflurane [146]. Thus, there is minimal evidence that serotonin receptors are mediators of anaesthetic action.

From neuroprotection to neurotoxicity: a spectrum of anaesthetic side effects

> `*It takes your enemy and your friend, working together to hurt you to the heart; the one to slander you and the other to get the news to you*.' – Mark Twain

The effect of anaesthetics is not limited to the desired short-term action in the operating room to allow surgery on patients. The ability of anaesthetics to produce immobility, amnesia and unconsciousness seem completely and quickly reversible. However, in addition to beneficial effects such as neuro- and cardioprotection, increasing concern over long-term detrimental effects such as neurotoxicity, neurodegeneration and postoperative cognitive dysfunction has recently arisen.

Neuroprotection

Much effort has gone into understanding the neuroprotective ability of anaesthetics in order to best use this advantageous aspect during neurosurgical anaesthesia. It is well established that inhalational agents such as isoflurane, and intravenous anaesthetics (propofol and barbiturates) provide protection from focal ischaemic and metabolic insults as confirmed by both in vitro and in vivo experimental models [147]. The exact mechanism of neuroprotection remains unclear, but its effects can remain for an extended period of weeks to months [148]. In vitro studies using rat hippocampal slice cultures verified the involvement of inhibitory $GABA_A$ and excitatory NMDA receptors in the global CNS protection afforded by volatile anaesthetics [149,150]. However, clinical trials using various blockers of NMDA receptors have failed to demonstrate benefit for global insults. A similar protective effect against cerebral ischaemia was found for the selective $alpha_2$-adrenoceptor agonist dexmedetomidine, which may involve the expression of the active focal adhesion kinase, a tyrosine kinase important for the adaptability and longevity of cells [151].

The molecular mechanism of volatile anaesthetic neuroprotection may also involve anaesthetic activation of K_{2P} potassium channels. Overexpression of the acid-sensitive K_{2P} channel TASK-3 in neurons of rat hippocampal slices protected them from a hypoxic and glucose deprivation injury [152]. Members of the TREK subfamily (TREK-1 and TWIK-related arachidonic acid-stimulated K^+channel (TRAAK)) are activated by the neuroprotective substances riluzole, polyunsaturated fatty acids and lysophospholipids and may contribute to neuroprotection by hyperpolarizing neurons during ischaemic insult [120]. Heurteaux *et al*. found that TREK-1 knockout mice were more vulnerable to kainic acid and pentylenetetrazol to induce seizures and spinal cord ischaemia than wildtype mice [122]. The molecular sites of action for both effects remain unknown. However, the involved channels overlap significantly with those that are among leading candidates for mediating the anaesthetizing effects of anaesthetics.

Neurotoxicity

Persistent detrimental effects of anaesthetics on CNS activity have also been identified recently. Postoperative cognitive dysfunction can be detected in approximately 25% of patients after one week and nearly 10% patients at three months following prolonged exposure to general anaesthetics. Advanced age, low education level, preoperative depression and the presence of postoperative pain, infection or respiratory complications contribute additional risk factors [153,154]. Additionally, animal studies have found decreased memory only in aged rats after anaesthesia with isoflurane [155] or nitrous oxide [156]. However, the interaction between anaesthetics and the process linked to the progression of Alzheimer's disease has not been established. Furthermore, two meta-analyses comparing rates of postoperative cognitive dysfunction in elderly patients undergoing general versus regional anaesthesia have failed to demonstrate a decreased incidence of cognitive dysfunction when administration of general anaesthesia was avoided [154,157].

In addition to the functional disruption of mental process, neurodegenerative changes can be detected in the brain of young organisms following exposure to anaesthetic drugs. A seminal observation was made by Jevtovic-Todorovic *et al*. who discovered that anaesthetic exposure to rodents within a particularly vulnerable developmental period overlapping the first postnatal week caused dramatic neurodegeneration within the hippocampus, a brain structure critical for memory [158]. A variety of anaesthetics, including isoflurane, ketamine, benzodiazepines and propofol have been found to cause neural injury. Based on this profile of damaging agents, it appears that both blockade of excitatory as well as potentiation of inhibitory (chloride) ion channels can induce injury to young neurons. The common denominator is depolarization of neonatal neurons. This apparent contradiction can be understood with the realization that the

chloride gradient is reversed in young neurons compared to adult neurons, so that the enhancement of GABA-ergic transmission causes chloride to leave the cell resulting in depolarization. Neurotoxic effects appear to follow this electrophysiologic effect.

The primate brain has also been shown to be susceptible to these toxic effects. The most recent data has been a retrospective examination of children exposed to anaesthetics, in which two or more exposures within the first two years of life was strongly associated with learning disabilities later in life [159]. The study of this alarming aspect of anaesthetic action is in the early stages but is being pursed with vigour because of its important implications.

Future research directions

`*A problem well stated is a problem half solved*' – Charles F. Kettering, inventor

Through the persistent efforts of a number of research groups around the world, the understanding of how anaesthetics work has advanced significantly in the last 25 years. Stricter criteria of what constitutes a legitimate site of action have been described and generally accepted. As a result targets that were once accepted as possible sites have been ruled out, and new potential candidates will have to fulfil these criteria to continue to demand research attention. However, an implicit assumption is that this definition of anaesthesia would be valid for all anaesthetics, but would simultaneously allow for the differentiation between various modes of action and effects [4].

The introduction of whole animal models, in which the receptors and channels that anaesthetics affect can be altered or eliminated, has produced additional insight. Targeted mutations in animals offer an elegant approach to *compare in vitro* observations with in vivo behaviour. Research that began with organisms in which genetic manipulation can be rapidly done in large numbers of organisms such as Caenorhabditis elegans [160] and Drosophila [161] to produce anaesthesia-sensitive or anaesthesia-resistant animals are increasing being done with knockout mice. These results underscore the power of using animal models in anaesthesia mechanisms research and how they will continue to allow major experimental advancements in the future.

Finally, at the molecular level, additional attention will be placed on the interplay between proteins and the lipid bilayer. For example, halothane molecules appear to group particularly in the upper regions of fatty acid chains and alternatively below the carbonyl group of the lipid. Thus, halothane induces a lateral expansion accompanied by a contraction of the lipid bilayer with consecutive changes in the electrical properties of the membrane [162]. At the same time, it is evidenced that halothane integrates with GABA receptors as well, where it causes increased inhibitory transmission [163].

Discovery of a single fundamental mechanism, which seeks to explain the whole of anaesthesia described above, has become less and less likely in recent years. The state of anaesthesia is more likely based on many different effects and on multiple molecular biological targets. These integrative thoughts sum up the `multiple target hypothesis'. In this sense, different anaesthetics produce various effects at different functional sites. These paradigms can be confirmed by computer models, which in the future may be useful in the design of new anaesthetics [164].

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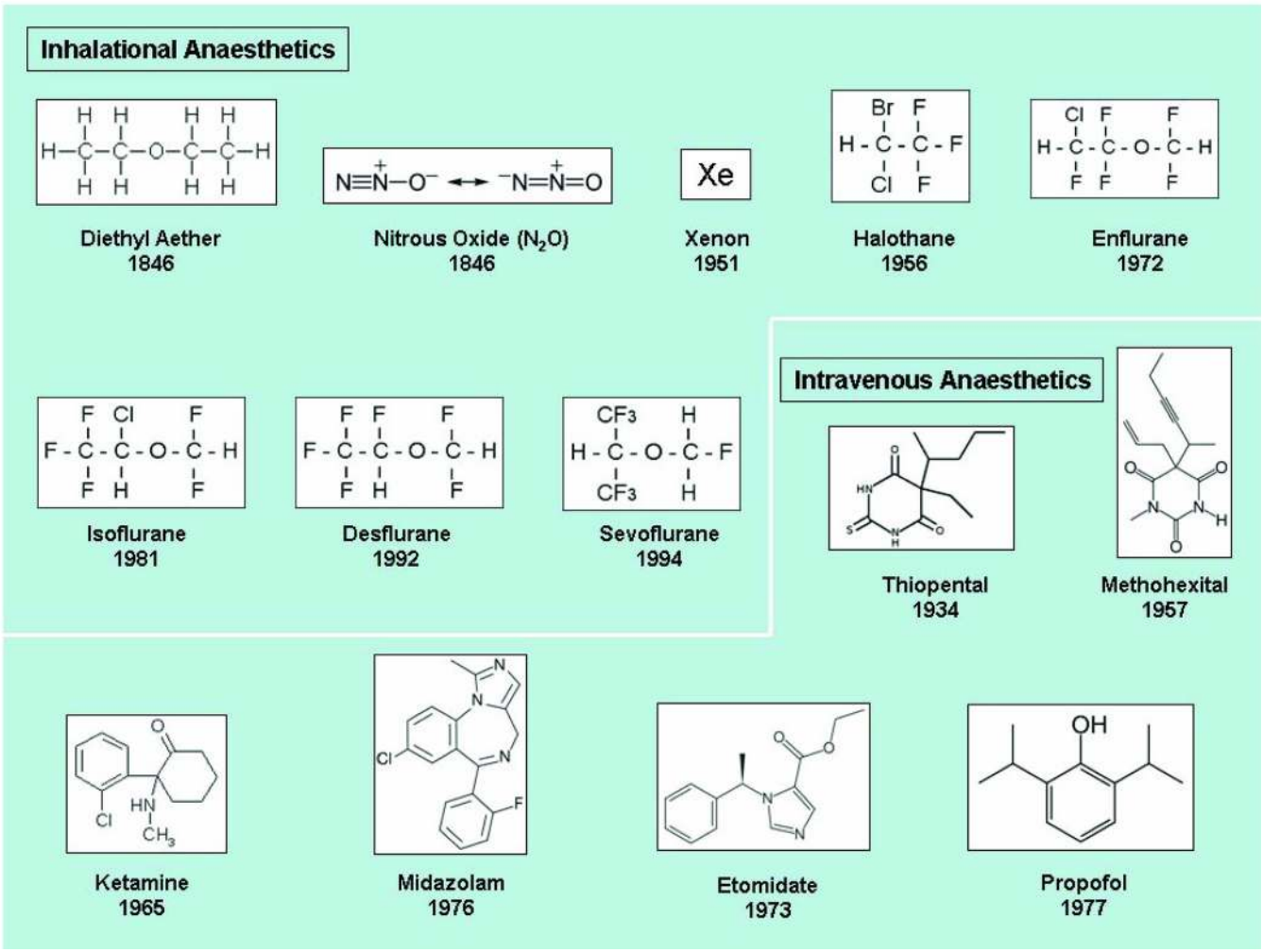

Fig. 1.
Overview of the most important, clinically used anaesthetics showing their chemical formulas and the year of their first clinical use.

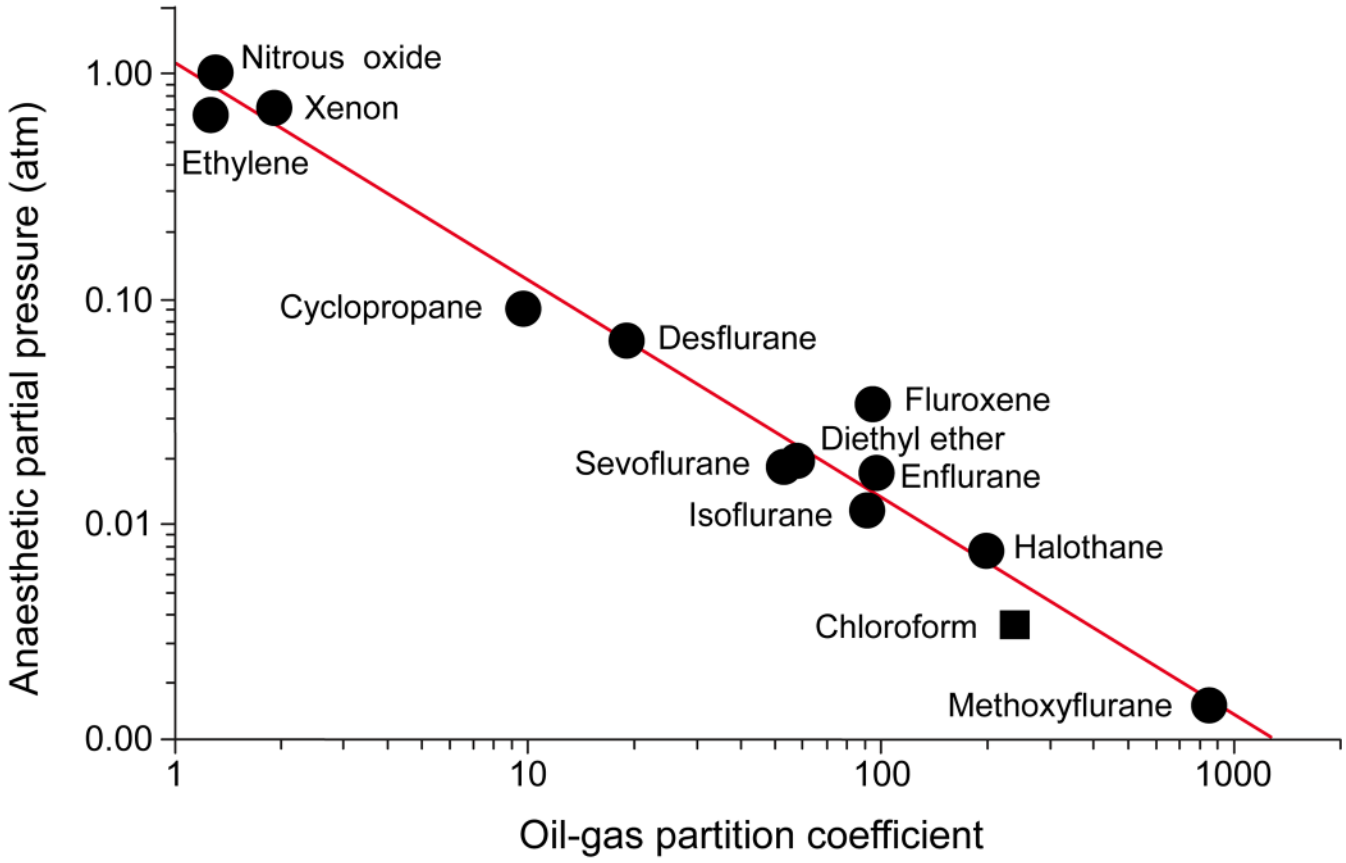

Fig. 2.

Meyer-Overton correlation. The partial pressure of inhalational anaesthetics that prevents movement in response to a surgical incision is plotted against the olive oil-gas-partition-coefficient showing the correlation between potency of an anaesthetic and lipophilicity (from Campagna JA *et al*. N Engl J Med. 2003; 348:2110–124, with permission).

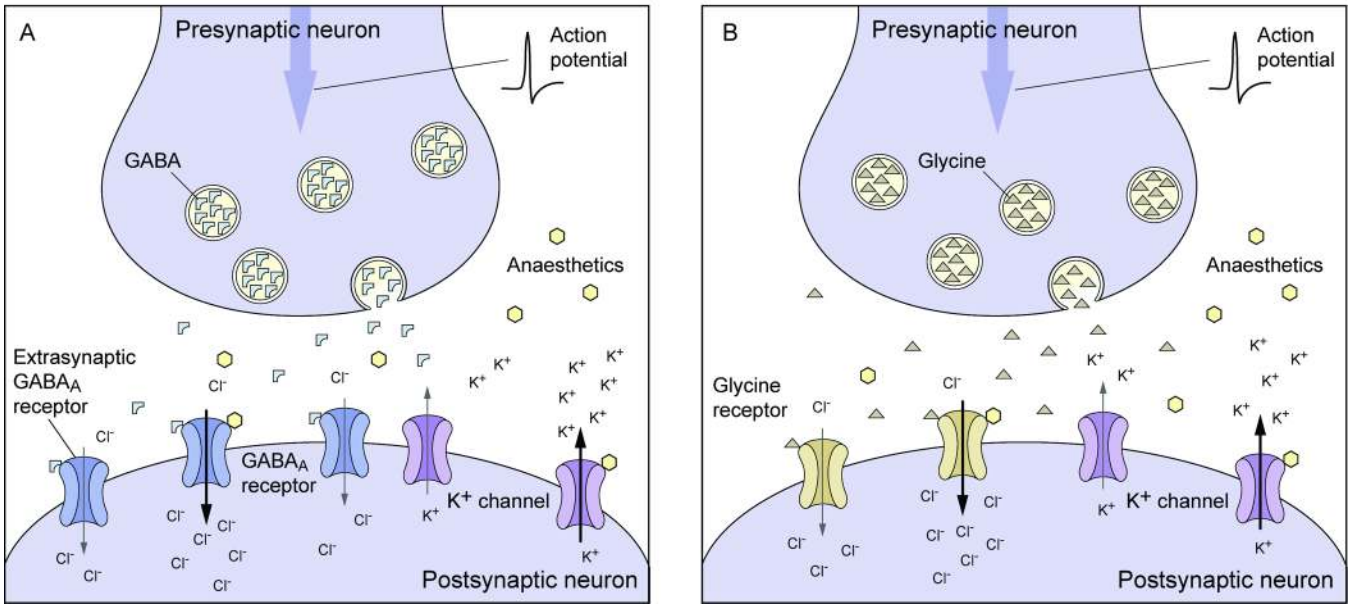

Fig. 3.

Effects of anaesthetics on inhibitory receptors and ion channels: currents passed by $GABA_A$-receptors (a), glycine receptors (b) and baseline potassium channels (a and b) are potentiated by anaesthetics. $GABA_A$ and glycine receptors allow primarily the influx of chloride ions leading to a hyperpolarisation of the cell. Baseline potassium channels also induce a hyperpolarisation of neuronal cells by an efflux of potassium ions. K+, potassium ions; Cl^-, chloride ions; inhalational anaesthetics (hexagons); GABA: gamma-aminobutyric acid.

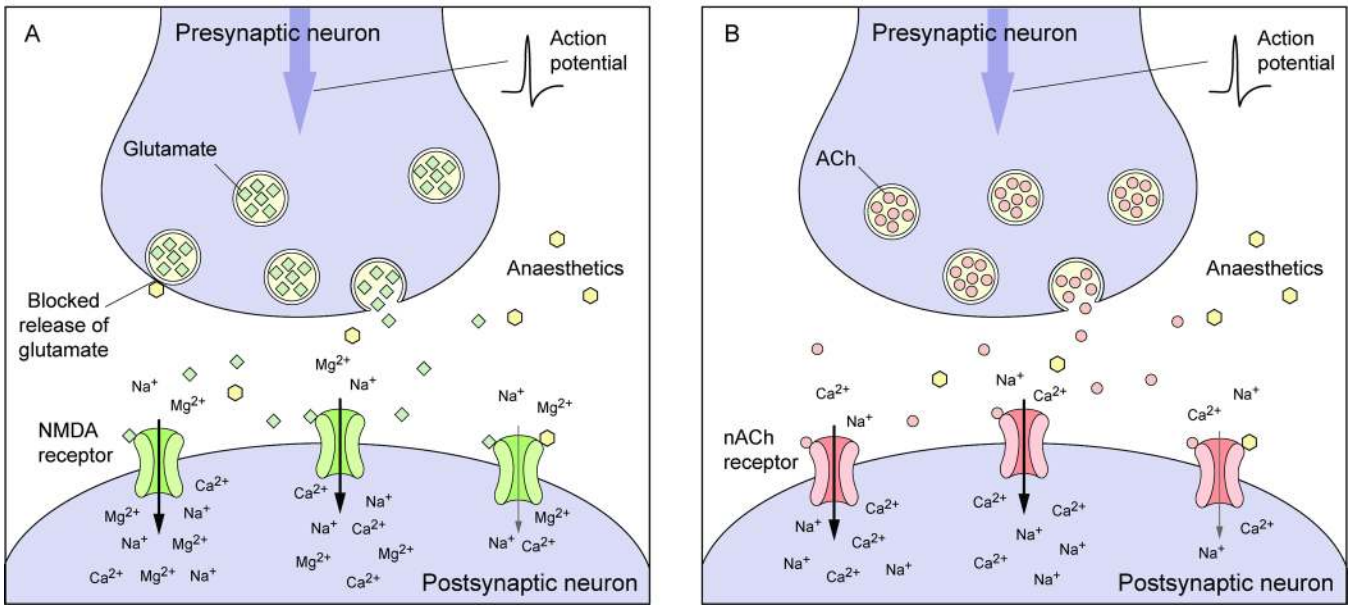

Fig. 4.

Effects of anaesthetics on excitatory ligand-gated NMDA/glutamate ion channels (a) and neuronal nicotinic acetylcholine receptors (b). These receptors are cation-selective, pass sodium, calcium and magnesium ions and are inhibited by anaesthetics. Volatile anaesthetics may also suppress the excitatory glutamatergic transmission via presynaptic inhibition of glutamate release. Na^+, sodium ions; Ca^+, calcium ions; Mg^+, magnesium ions; inhalational anaesthetics (hexagons); ACh, acetylcholine; NMDA-receptor, *N*-methyl-D-aspartate receptor; nACh-receptor, neuronal nicotinic acetylcholine receptor.

Table 1

Effects of various anaesthetics on ion channel/receptor targets

Anaesthetics	$GABA_A$	K_{2P} channel	Glycine	NMDA
[0,1–5]Intravenous anaesthetics				
Barbiturates	+	Ø	+	−
Propofol	+	Ø	+	−
Etomidate	+	Ø	+	Ø
Benzodiazepine	+	Ø	−	Ø
[0,1–5]Volatile anaesthetics				
Ether	+	+	+	−
Ether derivatives	+	+	+	−
Halogenated hydrocarbons	+	+	+	−
Ketamine	Ø	Ø	Ø	−
Nitrous oxide	Ø	+	+	−
Xenon	Ø	+	+	−

+, potentiating effect; −, inhibitory effect; Ø, no effect.

GABA, gamma-aminobutyric acid; K2P channel, two-pore potassium channel; NMDA, N-methyl-D-aspartate.

Table 2

Neurophysiological roles for putative protein targets of anaesthetics

Ion channel/receptor	Cellular function	Physiological and pharmacological effects
$GABA_A$ receptors	Increased chloride permeability	Anxiolysis
	Membrane hyperpolarisation	Sedation
	Decreased excitability	Amnesia
		Muscle relaxation
		Anticonvulsive action
Glycine receptors	Increased chloride permeability	Spinal reflexes
	Membrane hyperpolarisation	Scare, startle impulse
	Decreased excitability	Important inhibitory receptor at the spinal level
Nicotinergic acetyl-choline receptors	Increased permeability of monovalent cations and calcium	Memory
	Release of neurotransmitters	Nociception
		Mutations associated with epilepsy
		Autonomic functions
Glutamate receptors	Rapid excitatory impulse transmission	Perception
	Increased permeability of calcium, sodium and magnesium	Learning and memory
		Nociception
		Neuropathic pain
Voltage-gated potassium channels	Membrane repolarisation	Ubiquitously involved in basic neuronal function
	Spike frequency regulation	
	Shaping action potential	
ATP-sensitive potassium channels	Activated under conditions of metabolic depletion	Neuroprotection
		Insulin secretion
Baseline/background potassium channels (K_{2P})	Modulation of the resting potential and excitability	Neuroprotection
	Chemo- and mechano-sensitivity	Pain modulation
	pH sensitivity	Oxygen-chemoreception
		Few specific modulators known
Adenosine receptors	Change levels of intra-cellular second messenger cyclic AMP	Inhibit synaptic vesicle release
		Inhibitory modulation of NMDA receptors
Serotonin receptors	5-HTR_3: nonspecific cation conductance leading to membrane depolarisation	Neuronal excitation, emesis
	5-HTR_2: change in intra-cellular second messengers IP3 and DAG	Neuronal excitation

5-HTR, 5-hydroxytryptamine receptor; AMP, adenosine monophosphate; ATP, adenosine triphosphate; DAG, diacylglycerol; $GABA_A$, gamma-aminobutyric acid; IP3, inositol triphosphate; K_{2P} channel, two-pore potassium channel.