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Inhalational Anaesthetics: An Update on Mechanisms of Action and Toxicity

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Abstract

Inhalational anaesthetics have been used for induction and maintenance of general anaesthesia for more than 150 years. In human medicine desflurane, sevoflurane, and isoflurane are commonly used. All of them are fluorinated ether derivatives. Isoflurane is also chlorinated. Measurements of the expiratory gas fraction are used to guide anaesthesia via the MAC-concept. Specific partition coefficients outline the pharmacokinetic properties of inhalative anaesthetics. Mechanisms of action include specific interactions at numerous receptors of the central nervous system including inhibitory and excitatory circuits. The thesis of an unspecific interaction of inhalational anaesthetics with the cell membrane was abolished and is replaced by a theory describing a specific interaction between inhalational anaesthetics the cell membrane and a specific receptor. Organ toxicity of the modern inhalational anaesthetics is considered minimal. The impact of anaesthetics on brain development appears absent today. However, further studies are necessary to definitely answer this question.

Keywords: Volatile anaesthetics, Anaesthesia gases, Sevoflurane, Desflurane, Blood-gas coefficient

Introduction

For more than 150 years, inhalational anaesthetics are used for induction and maintenance of general anaesthesia. The so-called “balanced anaesthesia” describing the combination of an intravenous opioid and an inhalational anaesthetic for anaesthesia maintenance is the most common form of general anaesthesia nowadays. General anaesthesia is usually induced by intravenous application of a hypnotic agent (e.g., Propofol) in adults. In paediatric anaesthesia the inhalative induction of general anaesthesia is still a common procedure. In modern human medicine desflurane, isoflurane, and sevoflurane are the most frequently used inhalative anaesthetics.

History

Nitrous oxide was first synthesized in the 18th century and was regularly used in dental medicine from the mid of the 19th century because of its analgetic properties. The mid of the 19th century marks the beginning of modern anaesthesia. On the 16th of October 1846 William Morton successfully used ether to anesthetize a patient during the operative removal of a neck tumour. From that day on ether was more and more used for anaesthesia during operations and labour all around the world [1]. Only one year later in 1847 chloroform was used successfully for the same purposes. The successful uses of ether and nitrous oxide mark the beginning of modern anaesthesia. The currently used inhalative anaesthetics are all chlorinated and fluorinated derivatives of ether. Chloroform due to its toxic side effects on the heart and other inner organs is no more used in modern anaesthesia [2].

Physicochemical Properties

All the currently used inhalative anaesthetics are halogenated hydrocarbons. Preferred halogenation of the ether derivatives with fluorine leads to improved stability and pharmacokinetic properties. Toxicity is reduced by this procedure. Isoflurane is halogenated with fluorine and chlorine. Desflurane and sevoflurane are exclusively halogenated with fluorine. At room temperature isoflurane and sevoflurane are liquids. The boiling points of both

	Isoflurane	**Desflurane**	**Sevoflurane**
Structure	$C_3H_2OClF_5$	$C_3H_2OF_6$	$C_4H_3OF_7$
Relative molecular mass	184,5	168	200,1
Boiling point (°C)	48,5	22,8	58,5
Partition coefficients **Blood/Gas** **Fat/Blood**	 1,4 45,0	 0,42 27,0	 0,69 48,0
MAC_{50}-Values in O_2 (Vol-%)	1,15	6,0	2,04
Outline of the physical properties of the currently used inhalational anaesthetics.			

Table 1: Physicochemical properties.

agents are significantly above room temperature (48.5°C isoflurane and 58.5°C sevoflurane). Since the boiling point of desflurane is around room temperature (22.8°C) it must be stored in special non-breakable bottles and requires different vapor technology (Table 1).

Uptake and Distribution of Inhalational Anaesthetics

Inhalational anaesthetics are taken up via the lungs passively by diffusion. Thus, physical and respiratory factors determine the speed and amount of uptake and further distribution in the body. Uptake and distribution follow the rules described in Dalton's law of partial pressures. The sum of all partial pressures determines the total pressure of a mixture of gases. The partial pressure difference is the main driving force for redistribution from one compartment to another. Once the partial pressure is balanced between two compartments redistribution stops. After the uptake via the lungs inhalational anaesthetics are dissolved in the blood and transported via the bloodstream to the inner organs. The solubilities of inhalational anaesthetics in the blood and other tissues are described by the corresponding solubility coefficients for each inhalational anaesthetic. The most important coefficient is the blood gas partition coefficient. A high coefficient means that large amounts of the corresponding inhalational anaesthetic can be dissolved in the blood. Thus, equilibrium of inhalational anaesthetics between the alveolar space and the brain takes longer to be reached. Inhalational anaesthetics with a low blood gas partition coefficient are most favorable to use since controllability at induction and termination of general anaesthesia are better. The inhalational anaesthetic currently available with lowest blood-gas-coefficient is desflurane (0.42) followed by sevoflurane (0.69) and isoflurane (1.4). Redistribution of inhalational anaesthetics from the blood to fat tissue is a slow process that is particularly relevant during long lasting operations and procedures. Desflurane has the lowest fat blood partition coefficient among the currently available inhalational anaesthetics. Thus, desflurane has an exceptional good controllability during long lasting operations and is most suitable for obese patients [3].

MAC-Concept

Nowadays the measured concentration of an anaesthetic gas at end-expiration is used to guide inhalational anaesthesia. The anaesthetic potency of different inhalational anaesthetics is described by the so called minimal alveolar concentration (MAC). For certain states of the patient certain MAC-values are defined. MAC-values are inversely proportional to the oil gas partition coefficient. The most important MAC-Value is the MAC_{50} [4]. It describes the minimal alveolar (= expiratory) concentration of an anaesthetic gas where half of a defined group of exposed patients (no longer) reacts to a definitive surgical stimulus (skin incision) with a defense reaction. In a middle-aged healthy adult for isoflurane the MAC_{50} is 1.15 Vol%, for sevoflurane 2.04 Vol% and for desflurane 6 Vol%. Further important MAC-Values are the MAC_{awake} (50% eye-opening) and the MAC_{95} (95% no longer reacting to skin incision). Inhalational anaesthetics act synergistic regarding the MAC-Value. Thus, a second anaesthetic gas (e.g., nitrous oxide) can be added to lower the fraction needed of each inhalational anaesthetic for reaching the same MAC-Value [5]. MAC-Values are mostly dependent on the age of a patient. Sex, body height, and weight do not influence the MAC. MAC significantly decreases with age. The MAC is reduced by hypothermia, pregnancy, and anaemia. Central acting drugs like opioids lower the MAC as well [6,7]. Higher MAC-Values are needed in toddlers, full-term infants, and in patients with drug or alcohol-abuse.

Mechanisms of Action

Nowadays there is yet no comprehensive theory of how inhalational anaesthetics work. Only single aspects of the mechanisms of action are known. The MAC-concept is independent of a deeper insight into mechanisms of action but nevertheless clearly shows the dose-effect principle for inhalational anaesthetics.

Lipid-theory

The first theories about the mechanisms of action of inhalational anaesthetics were published in 1899 by Hans Meyer and two years later by Charles Overton. Both found out that there is a linear correlation between the oil gas partition coefficient and the anaesthetic potency of an anaesthetic gas. The findings were independent of the molecular origin of the described substances. The only relevant factor was the lipophilicity. They were able to describe the anaesthetic potency of ether, nitrous oxide and chloroform with the same theory. They concluded that there must be a unified mechanism of action for all inhalational anaesthetics. They proposed that all inhalational anaesthetics interrupted signal transduction at the lipid-bilayer of the cell membrane thus causing anaesthesia. Nowadays we know that the discovery made by Meyer and Overton is indeed somehow correct but for sure is not the key finding for a comprehensive theory on the mechanisms of action of inhalational anaesthetics. Lipophilicity, the ability to penetrate the central nervous system and store itself in a cell membrane alone are not enough to cause anaesthesia. It is well known that lipophilicity of a molecule increases with the length of its carbon chain, but today we know that its anaesthetic potency does not increase in the same way. When the carbon chain gets too long a molecule can totally loose its anaesthetic potency and can even reduce the anaesthetic potency of a concomitant applied anaesthetic agent [8]. Today we know that the anaesthetic potency of a substance can not only be correlated to its lipophilicity in a linear way but also e.g., with its ability to inhibit luciferase-activity [9]. The latter suggests a specific interaction with proteins and therefore suggests a way more complex mechanism of action than an unspecific interaction at the cell membrane.

Chirality

Besides the size of a molecule chirality seems to influence the anaesthetic potency. Isoflurane is a structural isomer of the no longer used enflurane and is usually provided as a racemic mixture. The anaesthetic potency of s-isoflurane is about 40% higher compared to r-isoflurane. Stereoselectivity is typical for interactions with the active center of a protein. It is not a typical trait for unspecific cell membrane interactions [10].

Today we have very tangible results suggesting modulation of various receptors of the nervous system by inhalational anaesthetics (Table 2).

Target	Binding-site	Effect	Comment
Cell membrane	Unspecific storage in the cell membrane	Disruption of signal transmission at the cell membrane	Part of the abolished lipid theory
$GABA_A$ receptor	A specific binding site at the $GABA_A$ receptor is suggested	Receptor response ↑ IPSP ↑	Variable effect Dependent on receptor subtype
Glycine receptor	Probably loop-2-region of the transmembrane domain	Receptor response ↑ IPSP ↑	---
Glutamate receptor	unclear Modulation of NMDA and AMPA channels	Receptor response ↓ EPSP ↓ Presynaptic release ↓	Variable effect Dependent on used inhalational anaesthetic
Nicotinic acetylcholine receptor	Several binding sites at the transmebrane domain Occludes channel pore	Receptor response ↓ Action potential ↓	---
TREK-1	Disruption of the cell membrane and release of lipid raft fragments	Lipid raft fragments → Activation of Phospholipase D2 → Activation of TREK-1	Activation of TREK-1 leads to unconsciousness

Overview of the currently known or suggested mechanisms of action of inhalational anaesthetics
IPSP: inhibitory postsynaptic potential; EPSP: excitatory postsynaptic potential

Table 2: Mechanisms of action of inhalational anaesthetics on different.

$GABA_A$ Receptor

The $GABA_A$ receptor is one of the most important inhibitory receptors of the central nervous system. It is the therapeutic goal of benzodiazepines, barbiturates, and various antiepileptic drugs. All those drugs modulate the $GABA_A$ receptor. Activation leads to a cellular influx of chlorine resulting in an inhibitory postsynaptic potential (IPSP). Isoflurane amplifies receptor response resulting in a larger postsynaptic inhibitory potential. The effect of isoflurane on different $GABA_A$ receptor subtypes varies. $GABA_A$ receptors with $\alpha_1\beta_1\gamma_2$-configuration show the greatest IPSP-augmentation through isoflurane. $\alpha_2\beta_1\gamma_2$ configured $GABA_A$-receptors show intermediate augmentation and $GABA_A$-ρ (formerly known as $GABA_C$) receptors are resistant to the effect of isoflurane. This reflects the fact that $GABA_A$-ρ-receptors are resistant to the effects of benzodiazepines and barbiturates as well [11]. It is clearly shown that the inhibitory postsynaptic potential is augmented by direct interaction of inhalational anaesthetics with $GABA_A$ receptors. The fact that the amount of amplification varies with receptor configuration suggests a specific binding site for inhalational anaesthetics at the receptor.

Glycine Receptor

Glycine receptors are like $GABA_A$ receptors widespread in the central nervous system harbouring inhibitory functions. Activation of glycine receptors also leads to a cellular influx of chlorine resulting in an inhibitory postsynaptic potential. Receptor response is augmented through isoflurane [11]. The glycine receptor seems to have a specific binding site for isoflurane. It is most probably located at the extracellular loop-2 region. By modulation of that region isoflurane induced receptor response can be enhanced [12]. Interestingly isoflurane seems to share its binding site at the glycine receptor with the local anaesthetic lidocaine.

The hypnotic properties of propofol and benzodiazepines seem to be mostly derived from their ability to modulate inhibitory postsynaptic potentials. In contrary to that inhalational anaesthetics not only modulate inhibitory circuits but seem to interact with excitatory circuits as well.

Glutamate Receptors

The most relevant excitatory neurotransmitter of the central nervous system is glutamate. It binds preferably to NMDA or AMPA receptors. Activation leads to a cellular influx of calcium thus leading to an excitatory postsynaptic potential (EPSP). Halothane can block NMDA and AMPA receptors thus hampering generation of an excitatory postsynaptic potential [13]. Ion currents through the NMDA receptor channel are lowered by sevoflurane [14]. The amount of released glutamate after depolarization is lowered by isoflurane and enflurane [15].

Today the mechanism of action of inhalational anaesthetics on excitatory neurons is not finally clarified. The inhibitory effects on presynaptic and postsynaptic sections vary in amount and effectivity with the used inhalational anaesthetic. It is not clear whether the inhibitory effect is caused through direct interaction with excitatory receptors and/or through a reduced presynaptic release of glutamate containing vesicles upon activation [16].

Nicotinic Acetylcholine Receptors

Inhalational anaesthetics seem to modulate nicotinic acetylcholine receptors as well. Isoflurane and sevoflurane lower the amplitude of action potentials in a dose-dependent reversible manner [17]. For isoflurane, several binding sites on the nicotinic acetylcholine receptor have been identified. Isoflurane binds in a dimer formation in the ion channel pore thus blocking the pore for ion currents. Hence the location of the binding sites suggests the mechanism of action. Additionally, isoflurane binds at three sites of the transmembrane domain and at the intracellular portion of the domain [18].

TREK-1

As described above inhalational anaesthetics modulate excitatory and inhibitory circuits of the central nervous system thus causing anaesthesia. Still, it is unclear how relevant the above-described single mechanisms really are and how they are orchestrated for anaesthesia. That this research field is still in progress is shown best by the fact that a recently published work presented a whole new mechanism of action for inhalational anaesthetics. This novel mechanism describes an interaction between cell membrane and receptors initiated by inhalational anaesthetics [19].

The cell membrane contains certain areas that are very rich in sphingomyelins, glycosphingomyelins and cholesterol. Those areas are called “lipid rafts”. By external impact, the conformation of the lipid rafts changes thus activating the phospholipase d2. The phospholipase d2 (PLD2) binds to the TWIK related potassium channel “TREK-1” activating the latter via binding phosphatidic acid to the receptor. Activation of the TREK-1 leads to a reversible loss of consciousness. The TREK-1 is not directly activated by inhalational anaesthetics but via an indirect mechanism. Inhalational anaesthetics store themselves in lipid raft areas thus leading to conformation changes and disruption of the lipid rafts. By this phospholipase d2 is activated and the activation chain for the TREK-1 is initiated. The importance of the TREK-1 for anaesthesia is

emphasized by the fact that a modification or the knock-out of the TREK-1 gene reduces susceptibility towards inhalational anaesthetics. Furthermore, the importance of the PLD2 for activation of the TREK-1 through inhalational anaesthetics is demonstrated in a mouse model. When the PLD2 is altered to be catalytic inactive, inhalational anaesthetics are not able to activate TREK-1 [19].

Organotoxicity

For elimination, the largest fraction of inhalational anaesthetics is not metabolized but just exhaled. Due to this elimination-mechanism inhalational anaesthetics are thought to be non-toxic. Potential hepato- or nephrotoxicity results from toxic metabolites generated during biotransformation. Around 2-5% of sevoflurane are metabolized in the liver. During that process large amounts of fluoride are generated and in a second step are eliminated via the kidneys. Older studies suggested potential nephrotoxic effects of the large amounts of generated fluoride. A temporary reversible decline of renal concentration ability is described for the no longer used inhalational anaesthetic methoxyflurane. The currently used inhalational anaesthetics do not seem to have any clinically relevant negative effects on renal function. Reactions of sevoflurane with CO_2-absorbers containing sodium, potassium and barium hydroxide can generate compound A. Compound A is nephrotoxic in a rat model, but nephrotoxicity of compound A could be ruled out for humans.

Hepatotoxicity with severe liver dysfunction could be observed after halothane-anaesthesia. During degradation of halothane in the liver relevant amounts of trifluoracetic acid (TFA) are generated. TFA functions as a haptene inducing the so-called autoimmune halothane hepatitis. There are rare case-reports describing a hepatitis occurring after exposition to desflurane [20]. Metabolization rate of desflurane is <0.1 % thus leading only to minimal amounts of trifluoracetic acid following [21].

Neurotoxicity of Central Acting Hypnotic Agents

Today the potential neurotoxicity of drugs used in the context of anaesthesia is strongly discussed. In 2016 the US Food and Drug Administration (FDA) warned for a prolonged (>3 hours) or repeated use of inhalational anaesthetics, benzodiazepines, and propofol for general anaesthesia in children younger than three years. The warning also included pregnant women in the third trimester. Concerns about negative effects of anaesthesia drugs on the brain function and development were the background of the warning [22]. Many experimental studies with the aim to examine potential neurotoxicity were carried out on newborn or pregnant animals. Regularly the tested animals were exposed to high levels of anaesthetics for very long times during the trials. Neuron apoptosis triggered by anaesthetics was one of the research goals in the carried-out studies. Furthermore, inhibition of neurogenesis or a changed form or function of neural synapses were examined. The latter could lead to a neuronal dysfunction or disturbed neurological development [23]. Today it is unclear whether the results from the animal experiments can be transferred to humans and if yes to what extent. Retrospective human cohort studies revealed controversial results. Repeated general anaesthesia due to the treatment of a hypoplastic left-heart syndrome during the new born stage and the preschool age revealed a worsened neurological outcome dependent on the amount of applied anaesthetics. Unfortunately, no differentiation regarding pre-existing genetic disorders or a low birth weight was made. The extent of the necessary operative procedures was not taken into consideration [24].

The 2019 published randomized controlled GAS-Trial (General Anaesthesia compared to Spinal anaesthesia) investigated the neurological outcome after general anaesthesia in children. The study included children who had to undergo hernia surgery before the 60th gestation week. At the age of 5, children who received general anaesthesia for less than one hour for hernia repair showed no deficits regarding neurocognitive development when compared to the regional anaesthesia group [25]. The recently published PANDA-Trial (Paediatric Anaesthesia and Neuro Development Assessment) revealed comparable result. This study compared siblings of whom one received general anaesthesia before the age of 3 and the other did not. No significant differences in neurocognitive development and behavior could be shown. The study rather hints that socioeconomic aspects (e.g., the level of education of the mother) influence child development [26].

The potential neurotoxicity of anaesthetics remains an unsolved question today. A meta-analysis investigating that topic showed that the until now carried out studies are lacking comparability and relevant cohort size. Study design usually runs short of being comprehensive enough to examine a such complex topic like brain-development (Table 3) [27]. It is not clear what clinical features anaesthesia-associated neurotoxicity has. Because of this lack of knowledge, it is most important to perform general anaesthesia for operations and procedures with the highest possible quality and safety standards. An unnecessary exposition must be avoided.

Conclusion

Inhalational anaesthetics have been used for more than 150 years to guide patients safely through an operation, intervention, or diagnostic procedure. A comprehensive theory of how inhalational anaesthetics work is not yet created. Single aspects are known showing complex

Study	Study design	Size of the study	Age at anaesthesia exposition	Result
GAS-Trial[25]	RCT; Comparison hernia repair under general anaesthesia v.s. spinal anaesthesia	**363** v.s.**359**	below **60th** gestation week	No difference regarding neurocognitive development
PANDA-Trial [26]	Cohort study; matched siblings, hernia repair under general anaesthesia	**105** sibling pairs	younger than **3** years of age	No difference regarding neurocognitive development
Glatz et al.[28]	National cohort study; all children born in Sweden	**33.514** (OP)v.s. **159. 619** (no OP)	younger than **4** years of age	Discrete difference regarding school grades (at the age of 16) and results in intelligence tests (at the age of 18) favoring control group. Clear difference regarding sex and parent´s level of education.
Hu et al.[29]	Retrospective cohort study	**463** (0 OP) v.s. **457** (1 OP) v.s. **116** (> 1 OP)	younger than **3** years of age	Association for learning disability and ADHS and (repeated) operations and general anaesthesia. It is emphasized that the study does not allow for conclusion whether operation or anaesthesia is causal for the findings.
O'Leary et al.[30]	Retrospective cohort study	28.366 (OP) v.s. 55.910	**38.6%** younger than **2** years of age **61,4%** between 2 and 5.7 years of age at time of operation	No difference when children were younger than 2 years at the time of operation. Association for older children between operation/ general anaesthesia and developmental disorder. Difference was discrete. No conclusion can be drawn whether operation or general anaesthesia is causal.
Selection of currently published trials investigating neurotoxicity and general anaesthesia				

Table 3: Human trials examining neurotoxicity in the context of general anaesthesia.

mechanisms of action. Today it is not known how those single aspects are orchestrated. Modern inhalational anaesthetics can be considered safe regarding organ toxicity. The impact of inhalational anaesthetics on brain development remains unclear today. Derived from the results of current studies it is more unlikely that general anaesthesia taken in account isolated from illnesses, operations and hospitalization has a negative effect on neurologic development.

Conflicts of Interest

Jan Jedlicka, Philipp Groene, Julia Linhart, Elisabeth Raith, Davy Mustapha, and Peter Conzen have no conflicts of interest.

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