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Carbonic anhydrase enzymes: Likely targets for inhalational anesthetics

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A B S T R A C T

Inhalational anesthetics such as isoflurane, desflurane and halothane are the mainstay medications for surgical procedures; upon inhalation, they produce anesthesia described as reversible unconsciousness with the features of amnesia, sleep, immobility and analgesia. To date, how they produce anesthesia is unknown. This study proposes that carbonic anhydrase enzymes are likely targets mediating the actions of inhalational anesthetics. Carbonic anhydrase enzymes, commonly expressed in living organisms, utilize carbon dioxide (CO_2) as a substrate and can generate H^+ and HCO_3^- from CO_2 with a great efficiency. There are remarkable lines of evidence for their likely roles in mediating anesthetic actions. Firstly, carbonic anhydrase enzymes are extensively expressed in the brain and spinal cord, and their importance in the brain activity, especially for the GABA and NMDA receptor signaling pathways, has been demonstrated in numerous studies. According to these studies, they provide HCO_3^- for GABA-A receptor activities and also buffer HCO_3^- excess resulting from NMDA receptor activation. Activation of GABA-A and inhibition of NMDA receptors are associated with the induction of anesthesia by the intravenous general anesthetics propofol and ketamine, respectively. Secondly, the carbonic anhydrase inhibitors topiramate and zonisamide are effectively used in the treatment of epilepsy for decades; their chronic use results in the requirement of increased levels of amobarbital in order to produce anesthesia in the epileptic patients during WADA test. In addition, given that CO_2 is a substrate for these enzymes, their tertiary structure is likely has a hydrophobic pocket suitable for the anesthetic molecules to bind. Inhalational anesthetic molecules, which are lipophilic and inert in nature, have an ability to cross the membranes and inhibit carbonic anhydrases, which might not be accessible by topiramate and zonisamide. Unlike carbonic anhydrase inhibitors, they could bind to the hydrophobic pocket for CO_2 molecules and produce a profound effect called anesthesia. Finally, there is a great deal of similarities between the physiological actions of inhalational anesthetics and carbonic anhydrase inhibitors; moreover well-known side effects of inhalational anesthetics could be associated with the inhibition of carbonic anhydrases. Therefore, this article presents a hypothesis that the anesthetic actions of inhalational anesthetics could be due to their inhibitory effects on the carbonic anhydrases. Investigating this hypothesis might lead to the development of new safer anesthetics, and more importantly it might reveal an endogenous anesthetic pathway, in which the carbonic anhydrase system is a component along with the GABA-A and NMDA receptor systems.

Introduction

Inhalational anesthetics such as halothane, isoflurane and desflurane are hydrophobic, small, and inert molecules with a 3-4-carbon backbone. Even though they are liquid at room temperature, they easily become gases due to their high vapor pressure; and their gaseous form is used for general anesthesia. These gaseous small molecules are inhaled from the lungs and carried to the brain and spinal cord through the blood. Their actions in the CNS produce reversible unconsciousness called anesthesia, which is one of the most important discoveries in the history of medicine.

How inhalational anesthetics produce all the effects of anesthesia including sleep, amnesia, immobility and analgesia has been a mystery since their discovery. There have been several theories to postulate their mechanism. According to Meyer-Overton lipid theory, their lipid solubility is crucial in their anesthetic efficiency; they act in the lipid membranes by increasing their fluidity, and therefore modulating the membrane proteins [1]. This theory was based on the data showing that there was a direct correlation between anesthetic potency and oil/water partition coefficient of an anesthetic. However, this theory falls short in explaining some actions of the anesthetic compounds. Firstly, a temperature elevation leading to a similar level of lipid perturbations does not cause anesthesia, in contrary, it increases the anesthetic concentrations required to induce anesthesia. Secondly, the lipid theory cannot explain 'the cut off effect' of the chemically homologous compounds in terms of their anesthetic action. The molecular size of a compound determines its ability for anesthetic action; having a molecular size larger than a certain point stops the homologous compound from being an anesthetic even though it has an ability to cause lipid perturbations. This fact also suggests that binding pocket size for anesthetic molecules is restricted. Finally, in vivo studies demonstrated that anesthetic potency of isoflurane showed stereo-selectivity, which suggests that specific interactions between binding pocket(s) and anesthetic molecules occur [1,2]. Subsequent studies steered the anesthetic research toward proteins and demonstrated that inhalational anesthetics could competitively inhibit proteins such as Firefly Luciferase and Ca-ATPase in clinically relevant concentrations [3–5] and bind to albumin [6]. These findings clearly showed that inhalational anesthetics could directly bind to proteins and affect protein function in the absence of lipids. Given the lipophilic properties of anesthetics and

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their ability to bind to proteins, it was consequently suggested that inhalational anesthetics bind to protein cavities, which are hydrophobic in nature, and modulate the functions of multiple proteins; therefore anesthetic action is mediated through multiple proteins [7,2]. However, it is unknown how the interactions between hydrophobic protein cavities and inhalational anesthetic molecules lead to anesthesia and what proteins are involved in the anesthetic actions; and thus targets for inhalational anesthetics are yet to be explored.

Even though the mechanism of action for inhalational anesthetics is not clear, the specific targets for intravenous general anesthetics such as barbiturates, propofol, and ketamine have been demonstrated. For instance, propofol [8–9] and barbiturates such as sodium thiopental induce anesthesia by binding to GABA-A receptors and enhancing its activation whereas ketamine acts through binding to NMDA receptors and blocking NMDA receptor activation [10–11]. Hence, the actions of intravenous anesthetics are primarily mediated by either enhancement of GABA signaling or inhibition of NMDA receptors [12]. It could be postulated that a fine balance between these two opposing pathways of inhibitory GABA and excitatory NMDA receptor signaling is essential in the functions of the brain, and they are important players in the pathway that leads to general anesthesia. In this context, the modulations of NMDA and GABA signaling by inhalational anesthetics have been shown [13–15], but how these modulations occur is not conclusive. Even though general consensus proposes that inhalational anesthetics bind to hydrophobic cavities of multiple proteins in a non-specific fashion, the possibility that they target a specific protein cannot be ruled out. Franks and Lieb proposed for specific protein targets for inhalational anesthetic molecules due to their ability to competitively inhibit Firefly Luciferase at physiologically relevant concentrations in their cell free systems. They also suggested that anesthetic molecules produce unconsciousness by selectively binding to a small number of proteins [3,16]. This present study further suggests the target for inhalational anesthetics could be a specific protein that plays an important role in the GABA and/or NMDA receptor signaling pathways; without the proper function of this protein the signaling from GABA and/or NMDA could be altered or disrupted. This protein target is not only a crucial component of the primary signaling pathways in the brain but is also able to interact with gaseous molecules. Could there exist such a protein? This article presents several lines of evidence supporting that this protein could be a group of enzymes called carbonic anhydrases. To date, the actions of inhalational anesthetics have never been linked to carbonic anhydrases. For the first time, this article focuses on carbonic anhydrases as likely gaseous anesthetic targets.

Evaluation of hypothesis

Carbonic anhydrase enzymes

Carbonic anhydrase enzymes (CA) are zinc-containing metalloenzymes that catalyze hydration and dehydration reactions of CO_2 and H_2CO_3, respectively; the hydration reaction generates bicarbonate (HCO_3^-) and hydrogen (H^+) ions from CO_2 whereas the dehydration reaction generates CO_2 from carbonic acid (H_2CO_3) as shown in the following reaction:

$$CO_2 + H_20 \overset{CA}{\leftrightarrow} H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

There are six classes of carbonic anhydrases: α, β, γ, δ, ζ and η; vertebrates express α-carbonic anhydrases whereas the other classes are expressed in different types of organisms. The different classes of carbonic anhydrases are structurally distinct and do not have significant sequence identity, but all of them contains a Zinc ion. To date 16 CA and CA-related protein (CARP) subtypes have been identified in human. The subtypes differ in tissue distribution and cellular location. CA-I, II, III, VII and XIII are cytoplasmic; CA-VA and CA-VB, mitochondrial; CA-IV and XV, glycosylphosphatidylinositol anchored proteins; CA-IX, XII, and XIV *trans*-membrane proteins; and CA-VI is secreted in the salivary. In addition, some subtypes such as CA-VIII, X and XI do not have the catalytic activity [17–18].

Carbonic anhydrases play an important role in the acid-base balance by providing an essential buffering system for the body fluids and tissues. They convert CO_2 that are produced by metabolic processes to H_2CO_3, which is transported in the blood through tissues. Lungs and kidneys are the essential organs in the buffering system. In the lungs, H_2CO_3 is converted to CO_2, which is eliminated through breathing whereas a fine balance between H^+ and HCO_3^- in the body fluids is established by elimination of excess H^+ and re-absorption of HCO_3^- from the kidneys. Besides pH balance, carbonic anhydrase is involved in a myriad of physiological functions including bone resorption, gastric acid production, renal acidification, gluconeogenesis, normal brain development, signal processing in the brain and memory formation [17].

Carbonic anhydrase inhibitors have been used in clinic for the treatment of various diseases including high altitude sickness, glaucoma and epilepsy. In addition, carbonic anhydrase inhibitors are under extensive research for their use in the treatment of obesity and cancer [19] while carbonic anhydrase activators have been studied for the treatment of memory disorders [20].

Carbonic anhydrase enzymes are commonly expressed in living organisms. Interestingly, there is uniformity in response to inhalational anesthetics within vertebrates; not only inhalational anesthetics can produce anesthesia in human and animals but also different classes of organisms such as plants, chordates, arthropods and nematodes have been shown be sensitive to inhalational anesthetics [21] suggesting that there is a common target for inhalational anesthetics within these different organisms. Carbonic anhydrases have the capability to be the common target because their functions are crucial for the survival of not only vertebrates but also other classes of organisms.

The importance of carbonic anhydrase in the activity of inhibitory and excitatory pathways in the brain:

Carbonic anhydrase is extensively expressed in the brain. In spite of the fact that the initial studies located the activity of carbonic anhydrase only in the neuroglia such as oligodendrocytes and choroid plexus [22–24], subsequent studies demonstrated the expression and activity of carbonic anhydrase in the primary [25–29] and central neurons [27,29–32]. Small, large and medium sized neurons in the spinal cord and dorsal ganglia and their axonal projections were found to have carbonic anhydrase activity [25,26]. Physiological and morphological studies further supported the existence of carbonic anhydrase activity in neuronal cell bodies of the cerebrum, cerebellum, medulla oblongata, and hippocampus [27,29–32] as well as the interstitial space in the CNS [33–34].

Distribution of the carbonic anhydrase subtypes throughout the CNS has been studied extensively. CA-VII was identified in the pyramidal cells of the hippocampus [31,35–36] using in situ hybridization and was suggested to play an important role in the high frequency stimulation of hippocampal interneurons and depolarization in the absence of glutamatergic excitation. In addition, CA-XIV was shown to be located in the axonal and neuronal membranes of the different parts of the human and murine brain including pons, medulla oblongata, hippocampus, cerebral cortex, cerebellum and corpus callosum. The CA-XIV [37] and CA-IV [33] activity was associated with the modification of the extracellular alkaline shift resulting from glutamatergic synaptic transmission. The importance of CA-IV and CA-XIV in the activity-dependent pH shifts was demonstrated in the study that absence of both proteins in the brain of the double knockout mice blocked the buffering ability in response to the alkaline shifts in the hippocampus [34]. CA-V, which is the mitochondrial form, was shown to localize throughout the nervous tissue of rats. Astrocytes and neurons were shown to express CA-V. In addition, CA-V in astrocytes was suggested to play an

important role in gluconeogenesis by providing bicarbonate for pyruvate decarboxylase while in neurons CA-V appeared to sequester Ca^{2+} in mitochondria and to play an important role in determining the level of intracellular Ca^{2+}. In addition, CA-V was associated with GABA-induced depolarization by the bicarbonate ion efflux [38]. The functional importance of carbonic anhydrase in the brain electrical activity was first shown with their ability to modulate GABA-A receptor channels and GABA-ergic signaling in the hippocampus [39].

GABA-A receptors are ion channels that are permeable to both Cl^- and HCO_3^- ions. Since membrane potential for Cl^- influx is greater than that for HCO_3^- efflux, GABA-A receptor activation leads to the Cl^- influx, which leads to membrane hyperpolarization and GABA-induced inhibition. However, sustained GABA-A activation dissipates the Cl^- influx and results in an increase of the HCO_3^- efflux, which leads to membrane depolarization and thus promoting NMDA receptor activation. Carbonic anhydrase plays a crucial role in the GABA-induced depolarization by providing HCO_3^-. Intracellular carbonic anhydrase generates HCO_3^- from CO_2 to sustain the HCO_3^- efflux whereas extracellular carbonic anhydrase converts HCO_3^- to CO_2, which in turn returns into the cell through the cell membrane and serves as a substrate for the intracellular carbonic anhydrase. Numerous studies have revealed that this mechanism plays an important role in signal processing, long-term synaptic transmission and attentional gating of memory storage in the hippocampus [39–41]. Therefore, the inhibition of carbonic anhydrase activity blocks GABA-induced depolarization and related NMDA receptor activation, and interferes with memory formation in the hippocampus. As a result, carbonic anhydrase activators have been suggested to enhance memory storage and formation; thus they are under investigation for the treatment of memory disorders such as Alzheimer's disease [20].

Besides the GABA-A receptor, NMDA receptor function is also modulated by the carbonic anhydrase activity. Stimulation of glutamatergic excitatory synaptic transmission by glutamate or electric stimulation evokes alkaline shifts in the hippocampus and cerebellum. The alkaline shifts result from H^+ influx, and excess HCO_3^- ion is buffered by the carbonic anhydrase activity. Acetazolamide, a carbonic anhydrase inhibitor, enhances the alkaline shifts [42,43] by blocking the carbonic anhydrase buffering. These studies clearly indicate the role of carbonic anhydrase in the regulation of neuronal pH and NMDA-induced excitation.

Another line of evidence proving that carbonic anhydrase plays an important role in the regulation of electrical activity of the brain, and their inhibition leads to depression of neuronal activity comes from effective use of the carbonic anhydrase inhibitors, topiramate and zonisamide in the treatment of epilepsy. In spite of the fact that mechanism by which they reduce epileptiform activity is unknown, the inhibition of carbonic anhydrase is clinically proven to regulate electrical activity of the brain. Therefore, these enzymes meet one of the plausibility criteria [1] for likely anesthetic targets.

Furthermore, the importance of carbonic anhydrase in the anesthetic actions of drugs comes from studies involving patients undergoing WADA test. It was reported that use of carbonic anhydrase inhibitors interfered with WADA test, which is used to detect the affected areas of the brain before brain surgery in patients with epilepsy [44–46]. In the test, amobarbital is injected in the carotid artery in one side of the brain and thus anesthesia is induced in that side. Epileptic patients who have been treated with a carbonic anhydrase inhibitor do not get anesthetized with amobarbital in the test. They need more amobarbital to induce anesthesia, indicating that the use of carbonic anhydrase inhibitors results in a right shift in the dose response curve of amobarbital, which acts by enhancing GABA-A receptor signaling. Even if the patients stopped using carbonic anhydrase inhibitors before the test, the same effect still existed, indicating that counter-regulatory alterations to the chronic use of carbonic anhydrase inhibitor leads to the right shift. If both of the carbonic anhydrase inhibition and GABA activation serve the same physiological outcome and/or carbonic anhydrase is a component involved in the function of GABA-A receptors, any changes in carbonic anhydrase activity would affect the action of GABA-A in respect with that outcome. In other words, compensatory changes-induced by chronic use of carbonic anhydrase inhibitors could physiologically antagonize the anesthetic actions of amobarbital.

Association of some adverse effects of inhalational anesthetics with the disruption of carbonic anhydrase activity

One of the prominent adverse effects of inhalational anesthetics is cognitive dysfunction [47–49]. Especially, elderly patients suffer from impairment of memory and decline in cognitive function post-surgery. Similar to inhalational anesthetics, topiramate and zonisamide, effective antiepileptic drugs with carbonic anhydrase inhibitory properties, have negative effects on cognition, and they have the greatest cognitive adverse effects including language impairment, word finding difficulties, speech impediment, decline in memory performance and mental slowing compared to other antiepileptic drugs. Due to their side effects, the ratio of patients who drops out the therapy is greater than that of patients using other antiepileptic drugs. These cognitive effects are reversible and disappear following withdrawal of the drug therapy [50–56]. Moreover, carbonic anhydrase enzymes play important roles in cognitive functions of the brain. For instance, the deficiency of CA-II enzyme in human causes mental retardation [57,58]. These data clearly demonstrate that both the use of inhalational anesthetics and inhibition of carbonic anhydrase activity by topiramate and zonisamide result in cognitive dysfunction.

Malignant hyperthermia is one of the adverse effects of inhalational anesthetics; the prevalence is 1/50000 among adult patients [59]. It is characterized with skeletal muscle rigidity, hyper-metabolic state, high fever and cellular ion imbalances due to the disruption of Ca^{2+} kinetics in the skeletal muscle. Briefly, calcium ion is essential for muscle function; its release from the sarcoplasmic reticulum (SR) into the cytoplasm initiates muscle contraction and activates muscle metabolism whereas muscle relaxation occurs through Ca^{2+} uptake into the SR. In malignant hyperthermia, the mechanisms for intracellular calcium release and uptake become dysfunctional. It is fatal unless treated with dantrolene, an antagonist for ryanodine receptors, calcium release channels. Malignant hyperthermia is associated with mutations in the ryanodine receptor and in some type of Ca^{2+} channels; the use of inhalational anesthetic in patients carrying these types of mutations leads to the onset of malignant hyperthermia. Interestingly, numerous studies have proposed that carbonic anhydrase plays important roles in the Ca^{2+} mobilization during excitation-contraction coupling by providing a rapid H^+ source as counter-ions for the Ca^{2+} release and uptake by the SR [60–63]. Blockade of carbonic anhydrase by a lipophilic carbonic anhydrase inhibitor in the rat skeletal muscle resulted in the retardation of force relaxation [64]. Moreover, Riley and Bain [25] proposed that carbonic anhydrases control the level of Ca^{2+} in the cytoplasm based on their studies performed with an electron microscopy. They demonstrated that carbonic anhydrase was located in the membranes of endoplasmic reticulum and mitochondria of rat peripheral sensory and motor neurons indicating their important roles in Ca^{2+} storage and release in these organelles. In addition, dichlorphenamide, a potent carbonic anhydrase inhibitor, is used in the treatment of periodic paralysis, which is a muscle disorder characterized by muscle weakness due to channelopathy induced-abnormalities in the excitation of muscle cells [65]. Furthermore, the use of topiramate increases muscle tone and produces muscle cramps in patients. Therefore, the disruption of carbonic anhydrase activity by inhalational anesthetics could explain the underlying mechanism by which inhalational anesthetics trigger malignant hyperthermia in patients with the dysfunctional Ca^{2+} kinetics.

Respiratory depression is one of the adverse effects of inhalational anesthetics. Carbonic anhydrases play crucial roles in every step of

breathing and respiratory control. Buffering function of carbonic anhydrases is a well-known phenomenon; the buffering of blood is mostly provided with the conversion of CO_2 into H^+/HCO_3^- by carbonic anhydrases located in the red blood cells. In turn, the concentration of H^+ and CO_2 in the blood and body fluids is very important in the activation of chemoreceptors located in the sensory cells, which detect O_2, CO_2/H^+ levels in the body fluids and play an essential role in the regulation of the respiratory centers in the brain stem. Central chemoreceptors are widely distributed within the brain stem whereas peripheral chemoreceptors are located in the carotid bodies. The firing of the central and peripheral chemoreceptors is affected by the carbonic anhydrase activity in the extra and intracellular milieu of the sensory cells [66–70]. Even though inhalational anesthetic-induced respiratory depression might result from the inhibition of the respiration-related neuronal networks, given that carbonic anhydrases are expressed in neurons, sensory cells and endothelial cells and are involved in CO_2 and pH regulation in the intracellular and extracellular milieu of the brain, it would not be unlikely that inhibition of carbonic anhydrase activity by inhalational anesthetics in the related CNS regions could disrupt the CO_2 hemostasis and contribute to respiratory depression.

Similarities between the physiological actions of carbonic anhydrase inhibitors and inhalational anesthetics

Inhalational anesthetics inhibit glucose-stimulated insulin secretion in humans and experimental animals [71–75]. Moreover, in isolated pancreatic islets isoflurane [76], halothane [77] and enflurane [78] were shown to inhibit glucose stimulated insulin secretion. In another report, isoflurane inhibited glucose-stimulated insulin release in rabbits and isolated rat pancreatic islets while propofol, an intravenous general anesthetic, failed to do so [79]. Interestingly, the rat and mouse pancreatic beta islet cells were found to have an intense CA-V expression, which was co-localized with insulin. The importance of the CA-V function in the insulin secretion was supported by the inhibition of glucose-stimulated insulin secretion by acetazolamide in the isolated pancreatic islets [80]. In a subsequent report, carbonic anhydrase activity in rat pancreatic islets was determined, and acetazolamide was found to inhibit glucose-stimulated insulin release, which was associated with the effects of acetazolamide on the membrane potential and cytosolic Ca^{2+} concentrations. Their findings suggest that CA-V plays an important role in glucose stimulated-insulin release in rat isolated pancreatic islets [81]. Neither carbonic anhydrase inhibitors nor inhalational anesthetics affect the glucose oxidation in the isolated pancreatic islets. Moreover, systemic administration of carbonic anhydrase inhibitors such as acetazolamide and dichlorphenamide may cause hyper/hypoglycemia in diabetic patients; these drugs should be used with caution for these patients. Thus, both classes of medication produce the inhibition of glucose-stimulated insulin secretion.

Inhalational anesthetics lower intraocular pressure and so do carbonic anhydrase inhibitors. Brinzolamide and dorzolamide are the topical carbonic anhydrase inhibitors, which are currently used for the treatment of glaucoma. Systemic administration of acetazolamide decreases intraocular pressure in human. Both of the systemic and topical administration decreases the production of aqueous humor, thereby lowering intraocular pressure [82]. Interestingly, inhalational anesthetics lower intraocular pressure in human and experimental animals as well [83–87]. Similar to carbonic anhydrase inhibitors, inhalational anesthetics were reported to decrease the rate of aqueous humor formation in dogs [88] and directly inhibit both electrical currents across the epithelium and aqueous humor production in the isolated rabbit iris-ciliary body [89].

Inhalational anesthetics and topical carbonic anhydrase inhibitors also share an eye-related adverse effect, which is dry eye. Inhalational anesthetics inhibit tear production in human and experimental animals [90–92]. In parallel, one of the side effects of the ophthalmic suspension of the carbonic anhydrase inhibitors, dorzolamide and brinzolamide, is dry eye.

Inhalational anesthetics induce vasodilation and increase the blood flow in some vascular beds especially in the brain [93–96]. Similarly, carbonic anhydrase inhibitor acetazolamide induces vasodilation in several organs and is known as a cerebral vasodilator [97–101].

Inhalational anesthetics produce central hypothermia by decreasing the thermoregulatory vasoconstriction, and they also decrease the thermoregulatory sweating in response to high temperatures. Therefore, inhalational anesthetics impair thermoregulatory response to heat and cold [102]. In comparison, carbonic anhydrase inhibitors affect the thermoregulatory response to some extent. Topiramate produces hypohidrosis, absence of sweating [103,104], and zonisamide oligohidrosis, diminished sweating, which might result in heat stroke in the patients whereas acetazolamide affects thermoregulation in response to exercise [105]. Topiramate was also reported to enhance the risk of hypothermia associated with valproic acid [106] while acetazolamide was reported to enhance hypothermic response produced by chlorpromazine [107]. Disruption in thermoregulation seems to be a common response to both inhalational anesthetics and carbonic anhydrase inhibitors.

Carbonic anhydrase is structurally suitable to be a target for inhalational anesthetics

CO_2, a gaseous molecule, is a substrate for carbonic anhydrases, which likely have a chemical structure to accommodate gaseous molecules, thus inhalational anesthetic molecules might target this protein. According to the current theory, gaseous anesthetics bind to protein cavities, which are hydrophobic in nature. The tertiary structure of carbonic anhydrase looks spherical. The active site is located in a large and deep conical shaped cleft that lies to the center of the enzyme. The Zn^{2+} resides at the bottom of the cleft. A hydrophobic pocket in the structure of CA-II was associated with CO_2 binding; the pocket was suggested to orient CO_2 molecules for the nucleophilic attack on the Zn^{2+} containing active site [108]. It is possible that the anesthetic molecules bind to this hydrophobic cavity of CA-II and the corresponding cavities in the other subtypes and prevent CO_2 molecules from binding to the active site, which, in turn, results in the enzyme inhibition. Indeed, chloral hydrate, a sedative-hypnotic drug, is a non-competitive inhibitor for CA-II [109]. Alternately, it is possible that inhalational anesthetics compete with CO_2 for binding to the hydrophobic domain and competitively inhibit the enzyme.

Consequences of hypothesis and discussion

Why carbonic anhydrase inhibitors do not produce anesthesia?

Even though carbonic anhydrase inhibitors can cause a diverse number of CNS effects including drowsiness, seizures, irritability, vertigo and confusion, hypoesthesia (numbness) and paresthesias (abnormal sensations) in the extremities and faces, they do not induce anesthesia. There could be several reasons for that.

Firstly, the physicochemical properties of carbonic anhydrase inhibitors determine the site of carbonic anhydrase inhibition, which, in turn, determine their diverse pharmacological effects. For example, carbonic anhydrases in the kidney are more prone to inhibition by conventional carbonic anhydrase inhibitors such as acetazolamide than those in other tissues since these drugs accumulate in the kidney, where they are eliminated from the body. This renal carbonic anhydrase inhibition by acetazolamide is a strong stimulus in the development of metabolic acidosis [110]. As a result, acetazolamide is very efficacious in the treatment of acute mountain sickness. However, acetazolamide has a limited ability to cross the blood brain barrier (BBB), which might contribute its weakness in the treatment of epilepsy. On the other hand, strong antiepileptic carbonic anhydrase inhibitors such as topiramate have lipophilic properties, which allow them to cross the BBB unlike

acetazolamide [111]. Additionally, the target for topiramate and zonisamide could be a membrane bound carbonic anhydrase subtype. Moreover, topical acetazolamide, unlike systemic acetazolamide, is not effective in the treatment of glaucoma because its physicochemical structure does not allow it to penetrate the cornea and reach an effective concentration in the site of action [112]. Therefore, the development efforts for topical carbonic anhydrase inhibitors yielded dorzolamide and brinzolamide, which are able to penetrate into the site of action. Compared to carbonic anhydrase inhibitors, inhalational anesthetics have completely different physicochemical properties, which may lead to their unique pharmacological profile. For instance, unlike carbonic anhydrase inhibitors, inhalational anesthetics can penetrate all the membranes of cells and organelles including endoplasmic reticulum (ER) and mitochondria due to their small size and lipophilicity. As a result, inhalational anesthetics could inhibit some specific carbonic anhydrase subtypes located in the ER, mitochondria or cytoplasm such as CA-V and CA-VII, which could not be reached by carbonic anhydrase inhibitors.

Secondly, the subtypes of carbonic anhydrase differ in their sensitivity to carbonic anhydrase inhibitors. For example, CA-II is more sensitive to carbonic anhydrase inhibitors than the membrane bound CA-IV and cytoplasmic CA-III [111]. It is possible that the sensitivity pattern of carbonic anhydrase subtypes for carbonic anhydrase inhibitors is different from that for inhalational anesthetics. Since inhalational anesthetic molecules are attracted to lipids, the membrane bound or mitochondrial subtypes of carbonic anhydrase could be more sensitive to inhalational anesthetics compared to the extracellular subtypes. This pattern change could also contribute to the profound effect of inhalational anesthetics.

Finally, the binding site for carbonic anhydrase inhibitors could be different from that for the anesthetic molecules. All of acetazolamide, topiramate and zonisamide contain a sulfonamide moiety in their molecular structures. The sulfonamide part replaces the hydroxyl/water molecule bound to the Zn^{2+} in the active site, which prevents CO_2 from a nucleophilic attack to the hydroxyl molecule and, in turn, inhibits the enzymatic reaction [18]. Inhalational anesthetics consist of lipophilic gaseous molecules, whose molecular structure is totally different from those of the carbonic anhydrase inhibitors. Therefore, their binding site could be hydrophobic part(s) of the enzyme. In turn, they may affect CO_2 interaction with the active site in a different manner and produce a unique pharmacological response.

Each one of these factors alone or all together could contribute to inhalational anesthetic-induced anesthesia and explain why carbonic anhydrase inhibitors do not cause anesthesia. Furthermore, it could be argued that the similarities between carbonic anhydrase inhibitors and inhalational anesthetics in terms of their physiological actions and side effects could be due to the off-target effects of the latter. On the other hand, the aforementioned physiological similarities occur in the clinically relevant concentrations of inhalational anesthetics. In addition, these similarities do not seem faint, but remarkable.

Rationale for endogenous anesthetic pathway

Inhalational anesthetics produce a profound pharmacological response in the body, which is reversible unconsciousness. The human body is also capable of undergoing reversible unconsciousness in traumatic events. People black out in fearful situations such as a car accident. After a bad car accident a person opens his eyes in an ambulance or a hospital room with tremors. He does not remember anything about the collision or how he got to the hospital room. When he is asleep, he does not feel the pain due to his broken bones or internal bleeding, and he cannot move. All these sleep, amnesia, immobility and analgesia due to the blackout are transient. This phenomenon suggests that the human body has a mechanism leading to blackout, which stops consciousness temporarily and allows the body to undergo deep sleep in response to traumatic events. This line of thought mandates the existence of a specific endogenous system, which inhalational anesthetics could activate when they are administered to patients.

Even though there is no known endogenous anesthetic ligand in the traditional sense, the modulation of GABA and NMDA receptors are known to be capable of producing reversible unconsciousness. On the basis of evidence presented in this paper, it is not irrational to suggest that carbonic anhydrase enzyme might be an important component in the endogenous anesthetic pathway. How could the inhibition of carbonic anhydrases lead to reversible unconsciousness? These enzymes are all about ions: they provide the fastest production of H^+ and HCO_3^-, which are involved in the fine acid/base balance and the movement of other ions including K^+, Cl^- and Ca^{2+} among cell compartments such as intracellular, extracellular, ER and mitochondria through co-transportation or counter-transportation. The movement of H^+, HCO_3^- and other ions across the membranes is crucial for the functions of neurons such as neurotransmitter release and signal transmission. The inhibition of carbonic anhydrases could disrupt the ionic balance in the specific compartments of the neuronal networks and consequently could modulate the activity of multiple proteins such as GABA-A, NMDA receptors and K+ channels. This could result in the inhibition of neuronal function, which might disable the body to detect and respond to noxious stimuli.

Interestingly, the dose response curve of inhalational anesthetics in human is very steep. This steepness was suggested to be due to the binding of the anesthetic molecules to multiple proteins, thereby modulating them; the anesthetic response is produced as a result of the partial contributions from these multiple functional modulations [7]. In this context, the inhibition of carbonic anhydrases results in the indirect modulation of multiple proteins such as GABA-A and NMDA receptor channels and K^+ channels, which could also underlie the steepness of the anesthetic dose response curve. Alternatively, the steepness could be caused by unconventional interactions among CO_2, inhalational anesthetics and hydrophobic pockets of different subtypes of carbonic anhydrase enzymes in the CNS.

How to investigate the hypothesis and conclusion

In order to investigate the carbonic anhydrase hypothesis, inhalational anesthetics could be initially studied for its effects on the activity of carbonic anhydrases in the cell free systems as well as in the cell extracts. Physiologically relevant concentrations of isoflurane, halothane and sevoflurane could be tested for their ability to inhibit the recombinant forms of different subtypes especially the mitochondrial subtype CA-V, cytoplasmic CA-VII and membrane proteins CA-XIV and CA-IV, all of which are expressed in the brain. Also, the stereo-selectivity of the inhibition could be determined by using different isomers of isoflurane. Once the specific inhibition of carbonic anhydrase enzymes by inhalational anesthetic agents is established and characterized, the anesthetic binding site of the enzyme could be determined. Furthermore, the activity of CA enzymes could be measured in the cell extracts and in vivo in the presence and absence of inhalational anesthetics. If the in vitro inhibition of the enzyme by the anesthetics shows a competitive nature, the dose response curve of the anesthetics could be studied in the presence of increasing concentrations of CO_2 in vivo. A right shift in the dose response curve in the presence of increasing concentrations of CO_2 further confirms a competitive inhibition of the enzyme by the anesthetics, which also associates their anesthetic effect with carbonic anhydrase inhibition. Of course, CO_2 concentrations to be used in these experiments have to be below toxic level. Furthermore, any change in the inhibitory potency of inhalational anesthetic in the presence of carbonic anhydrase inhibitors such as topiramate and zonisamide could be measured in vivo and in vitro in order to further characterize the anesthetic-induced inhibition of the enzymes.

Results from the aforementioned experiments would be very valuable. If the data supports the theory, not only the role of carbonic

anhydrase enzymes in the anesthetic actions would be proved, but also interactions among the carbonic anhydrases, GABA inhibitory and NMDA excitatory pathways could be determined. This information would reveal an endogenous anesthetic pathway and also help discover new anesthetics with a good side effect profile. In addition, the myocardial protective effect of inhalational anesthetics against ischemia reperfusion injury is a well-known phenomenon [113]. Identification of anesthetic targets would lead to the development of effective medications to treat ischemic heart diseases. If the data does not support the theory, this study would still be valuable. It would bring a unique approach in terms of investigating the mechanism of action for inhalational anesthetics, which is the possibility for specific inhalational anesthetic targets and for an endogenous anesthetic pathway. Moreover, in case the similarities between the physiological actions of inhalational anesthetics and carbonic anhydrase inhibitors are found to be due to off-target effects of the former, then the data could provide information regarding the protein-binding site of the anesthetic molecules.

In summary, the carbonic anhydrase hypothesis presented in this paper has the capability to explain many peculiar aspects of the actions of inhalational anesthetics, among which are their required lipophilic properties, their ability to bind to proteins and inhibit enzymes, the uniformity of the anesthetic response within different organisms and the possible involvement of multiple proteins in the anesthetic actions. Taken together with the physiological and pharmacological evidence, this hypothesis warrants an investigation for the roles of carbonic anhydrases in mediating the actions of inhalational anesthetics.

Conflict of interest

None.

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