Are neuronal voltage-gated calcium channels valid cellular targets for general anesthetics?

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Abbreviations: CNS, central nervous system; DH, dorsal horn; DRG, dorsal root ganglia; GABA, γ-aminobutyric acid; KO, knock out; LORR, loss of righting reflex; MAC, minimum alveolar concentration; NMDA, N-methyl-D-aspartate; nRT, nucleus reticularis thalami; HVA, high voltage-activated; LVA, low voltage activated; PNS, peripheral nervous system; WT, wild-type

The effects of anesthetics and analgesics on ion channels have been the subject of intense research since recent reports of direct actions of anesthetic molecules on ion channel proteins. It is now known that ligand-gated channels, particularly γ-aminobutyric acid (GABA) and N-methyl-D-aspartate (NMDA) receptors, play a key role in mediating anesthetic actions, but these channels are unable to account for all aspects of clinical anesthesia such as loss of consciousness, immobility, analgesia, amnesia and muscle relaxation. Furthermore, an assortment of voltage-gated and background channels also display anesthetic sensitivity and a key question arises: What role do these other channels play in clinical anesthesia? These channels have overlapping physiological roles and pharmacological profiles, making it difficult to assign aspects of the anesthetic state to individual channel types. Here, we will focus on the function of neuronal voltage-gated calcium channels in mediating the effects of general anesthetics.

Introduction

Based on the membrane potential at which they activate, calcium channels are subdivided into high voltage-activated (HVA) and low voltage-activated (LVA) or transient (T-type) Ca2+ channels. Neuronal channel subtypes have central functions in sensory, cognitive and motor pathways by controlling cell excitability and neurotransmitter release. Pharmacological and physiological experiments support the existence of multiple types of native HVA Ca2+ channels.1 These channels are products of different genes, which give rise to α1 subunits that form the pores of the Ca2+ channels, namely the CaV1 family (former α1S, α1C, α1D, α1F) encoding L-type; CaV2.1 (α1A) encoding the P/Q-type; CaV2.2 (α1B) encoding the N-type; and CaV2.3 (α1E) encoding the R-type. Similarly, cloning of the T-type calcium channels have revealed that they consist of three main isoforms, CaV3.1 (α1G),2 CaV3.2 (α1H)3 and CaV3.3 (α1I).4 Associated with the pore-forming α1 subunit of HVA channels is the membrane-anchored, largely extracellular α, δ, the cytoplasmic β and sometimes a transmembrane γ subunit; these subunits dramatically influence the properties and surface expression of these channels.5,6 In contrast, association of ancillary subunits with T-type channels in native tissues has not been conclusively demonstrated. Earlier in vitro studies have indicated that some classes of voltage-gated calcium channels expressed in neurons and neuroendocrine cells are affected by volatile general anesthetics at clinically relevant concentrations.7–11 However, the possible functional significance of these effects of anesthetics is not well studied.

General Anesthetics

In the brain, research has focused on the effects of anesthetics on areas involved in cognition, memory, learning, sleep and attention. The parietal and prefrontal association cortices are affected at low doses of anesthetics, causing amnesia and learning deficits. In contrast, activity in the reticular formation and thalamus is suppressed only at anesthetic concentrations that cause unconsciousness.12 The thalamus is a major ‘gateway’ of functional connections between the cortex, thalamus and spinal cord that are essential for awareness and dampened during the anesthetic state.13 Sleep, which shares many features with general anesthesia, is also regulated in the thalamus, making it a likely target for anesthetics.

Both inhaled and intravenous agents rely on ion channels to mediate their anesthetic and analgesic effects in the brain and spinal cord, while peripheral channels are likely to be responsible for various side effects. Volatile inhalation anesthetics (e.g., isoflurane, halothane, sevoflurane, desflurane) are a closely related group of agents that have similar mechanisms of action. They are thought to exert their inhibitory effects in the central nervous system mainly via potentiation of function of GABA receptors which causes influx of chloride ions into postsynaptic neurons and subsequent hyperpolarization of the cell membrane.14

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Similar mechanisms of neural inhibition via GABA_A receptor agonists are proposed for intravenous anesthetics such as propofol (diisopropyl alcohol), etomidate (ethyl 3-[(1/R)-1-phenylethyl] imidazole-4-carboxylate) and various barbiturates. In contrast, the clinical effects of another widely used anesthetic gas, nitrous oxide (laughing gas, N₂O), and an intravenous agent, ketamine, are thought to be largely mediated by the inhibition of NMDA receptors. Indeed, at clinically relevant concentrations both N₂O and ketamine have very little effect on GABA_A-gated ionic currents in vitro. While GABA_A and NMDA receptors explain many anesthetic effects, they do not provide a complete explanation for differences among various agents in their clinical applications. Anesthetic agents have the ability to interact with many other ion channels, which may fill the gaps in our understanding. These channels include voltage-gated sodium, potassium and calcium channels, as well as nicotinic acetylcholine receptors and glycine receptors. In this review, we will focus on evidence supporting neuronal voltage-gated calcium channels as a target for anesthetic agents.

**High Voltage-activated Calcium Channels**

High voltage-activated calcium channels have consistently shown sensitivity to volatile anesthetics in both oocytes and mammalian heterologous systems. These studies have shown that isoflurane and halothane at least partially inhibit L-, N-, P/Q and R-type channels at clinically relevant concentrations by increasing channel inactivation and decreasing recovery from inactivation. However, anesthetic sensitivities of voltage-gated calcium channels in native neurons are not well studied.

L-type channels appear to be needed for the inhibitory effects of isoflurane in hippocampal neurons. Data also suggest that volatile anesthetics interact directly with L-type channels and interfere with channel modulation by G-protein coupled receptors. This family of HVA channels is also present at high densities in the heart, which may result in the negative inotropic effects that these volatile anesthetics possess. L-type channels in human myocardium have been shown to be blocked by halothane and other fluorinated anesthetics block at low subanesthetic concentrations.

Various research groups have reported inconsistent effects of prototypical general anesthetics on N-, R- and P/Q-subtypes of voltage-gated calcium channels. For example, recombinant N-type channels expressed in mammalian cell lines are reported to be only slightly inhibited (<14%) by clinical concentrations of isoflurane (320 μM), with no measurable effects with xenon application. In contrast, at these concentrations, isoflurane inhibited about 60% of peak N-type currents in human neuroblastoma cell line. Hall and colleagues found P/Q-type channels from rat Purkinje neurons to be largely insensitive (inhibitions of <10%) to a variety of inhalation and intravenous anesthetic agents, including halothane, isoflurane, thiopental, pentobarbital, propofol and ethanol. Similarly, an earlier study found only 10% current inhibition of recombinant human CaV2.3 currents in mammalian cells with clinically-relevant concentrations (100 μM) of pentobarbital.

Others have reported greater state and use-dependent inhibition of recombinant human P/Q-type currents with clinically relevant concentrations of pentobarbital (up to 50% current inhibition with 100 μM) in a heterologous system, but differences in cell type, presence of ancillary calcium channel subunits and charge carrier make comparison difficult. In some instances, actual concentrations of volatile anesthetics in vitro were not measured making meaningful evaluations between different preparations even more difficult.

A recent study demonstrated that R-type channels contribute to the inhibitory effects of isoflurane in the rat thalamus. In this study authors used both in vitro patch-clamp recordings of native and recombinant currents as well as in vivo EEG recordings from wild type (WT) and CaV2.3 knockout (KO) mice. Major findings of this study are that isoflurane at clinically relevant concentrations (IC₅₀ 240 μM) inhibited native calcium currents in nucleus reticularis thalami (nRT) neurons and recombinant CaV2.3/β3 currents. Furthermore, inhibitory effects of isoflurane upon CaV2.3-dependent GABAergic synaptic transmission in nRT neurons are confined to the presynaptic targets. Ensuing in vivo studies demonstrated that inhaled isoflurane at equivalent concentrations in vivo had substantially smaller depressant effects on EEG signalizing in CaV2.3 KO mice when compared to WT littermates. R-type (CaV2.3) channels are capable of opening with small depolarizations in comparison to other HVA channels. Because the thalamus has a key function in processing sensory information, sleep and cognition, modulation of its GABAergic tone by presynaptic R-type Ca²⁺ channels may contribute to the clinical effects of general anesthesia.

**Low Voltage-activated Calcium Channels**

Low-voltage-activated or T-type calcium channels activate with small depolarizations and allow calcium influx at resting potentials, so that small differences in channel activity can result in large changes in cellular excitability and/or second messenger pathways. These channels are located throughout the spinothalamic pathway, where nociceptive information passes from peripheral sensory neurons to the cortex (Fig. 1). T-channels in small-sized dorsal root ganglia (DRG) neurons are thought to function in pain signaling. Small and medium DRG neurons contain both CaV3.1 and CaV3.2 channels, though CaV3.2 predominates. CaV3.2-null mice have significantly decreased responses to acute somatic and visceral pain. Furthermore, oligonucleotide antisense studies against CaV3.2 reported similar results. This evidence makes it clear that peripheral CaV3.2 T-channels are pro-nociceptive in the DRG.

In vivo studies have shown that anesthetic-induced loss of movement in response to pain is mediated primarily in the spinal cord. Actions in the brain are not apparently critical to inhibit motor responses to pain. This has been demonstrated in anesthetized rats, where cervical transection of the spinal cord did not change the minimum alveolar concentration (MAC) for painful limb stimulation. Furthermore, in situ studies have indicated that all three isoforms of T-type channels are expressed predominantly in the dorsal horn of the spinal cord, an important pain pathway.
processing region of CNS. Thus it is likely that any contributions of T-channels in the spinal cord to MAC of general anesthetics are indirect, since the effects would be on nociceptive pathways rather than motor pathways in the spinal cord.

T-type channels are also expressed in various brain regions and are particularly abundant in thalamic nuclei, where they are crucial for control of the functional states of those neurons. Specifically, CaV3.1 is mainly expressed in thalamo-cortical relay neurons, while CaV3.2 and CaV3.3 are expressed in nRT neurons, the main inhibitory structure in the thalamus. Inhibition of thalamic processing of sensory information has been recently implicated as a possible contributor to clinical effects of anesthetics such as loss of consciousness and sedation. Therefore, the potential of T-channel isoforms expressed in the thalamus, spinal cord and DRG as targets for the action of general anesthetics remains an important issue in our understanding of the cellular mechanisms of anesthetic action.

It has been previously shown that isoflurane, at clinically relevant concentrations, similarly inhibits recombinant and native T-current variants in peripheral and central neurons. N2O has been shown to specifically affect the CaV3.2 isoform, while isoflurane and halothane affect all T-type CaV3 channel isoforms equally. On the other hand, it appears that intravenous general anesthetics such as propofol, etomidate and ketamine do not significantly inhibit native T-type channels at clinically useful concentrations. One exception among intravenous anesthetics is pentobarbital which at clinically relevant concentrations inhibits about 20% of DRG T-currents and about 30% of general anesthetics is pentobarbital which at clinically relevant concentrations (up to 80%), nitrous oxide’s block of T-type calcium current is specific while CaV3.2 and CaV3.3 are expressed in nRT neurons, the main inhibitory structure in the thalamus.

In the CNS, these include L-type, N-type, P/Q-type, R-type and T-type (CaV3.1, 3.2, 3.3) channels. Cav3.2 T-type channels are also sensitive to anesthetics in the PNS. Examples of T-type channel-specific neuroactive steroids that are devoid of effects on GABAA receptors such as ECN are able to alleviate chronic constriction injury-induced thermal hyperalgesia, suggesting that peripheral T-type channels may mediate some N2O analgesia. This strongly suggests that at least part of the potent N2O-induced analgesia could be mediated by blockade of CaV3.2 T-type CaV3 channels. This finding was corroborated by recent in vivo experiments using formalin test of inflammatory pain in mice. Interestingly, authors found that inhibitory effects of N2O upon native and recombinant CaV3.2 currents in vitro and N2O analgesia in vivo were dependent upon presence of trace metals and free oxygen radicals. Furthermore, N2O-mediated analgesia was significantly diminished in CaV3.2 KO mice when compared to WT littermates. These data directly implicate CaV3.2 T-type channels in analgesic effects of N2O, at least in one commonly used inflammatory paradigm.

Previous reports indicate that both thalamic and global knockouts of the CaV3.1 gene lead to fragmentations in sleep patterns and thus would suggest possible implications for the sedative/hypnotic effects of general anesthetics. Interestingly, mutant mice with global knock-out of the CaV3.1, and CaV3.2, isoforms of T-type channels displayed significant alterations in anesthetic end points. While neither of these mutant mouse lines displayed alterations in LORR (loss of righting reflex), a test routinely used to assess hypnotic effects of general anesthetics, these studies reported delayed anesthetic induction with CaV3.2 knockout mice similar to CaV3.1 knockout mice. These findings are somewhat surprising but emphasize the role of these isoforms in hypnotic/sedative effects of isoflurane and suggest that a sleep phenotype of CaV3.2 KO mice should be studied in the future.

Based on the fact that CaV3.2 isofrom of T-channels is strongly implicated in nociception, it is unsurprising that anesthetic agents, which inhibit T-channels, such as isoflurane, also often possess notable analgesic qualities. In agreement with this, we report decreases in MAC requirements for isoflurane in CaV3.2 KO mice, likely resulting from the prominent role of these channels in pain signaling. In contrast, studies of CaV3.1 KO mice did not find any changes in MAC for isoflurane or other volatile anesthetics. Although both CaV3.1 and CaV3.2 isoforms are similarly inhibited by isoflurane, and are expressed in central and peripheral pain pathways, it appears that CaV3.2 is a more important contributor to anesthetic-induced analgesia. These findings also underscore...
the consideration that any future pharmaceutical development of drugs that selectively inhibit Ca₃.2 T-channels may also be a useful adjuvant for general anesthesia.

Conclusion

Voltage-gated calcium channels are expressed in many of the locations that anesthetics are thought to target, such as the brain, particularly the thalamus, spinal cord and sensory neurons (Fig. 1). These channels are also functionally plausible targets for general anesthetics: HVA channels have been strongly implicated in synaptic transmission, while LVA channels are required for burst firing in the thalamus and are pronociceptive in the periphery. L-, R- and T-type channels are significantly inhibited by various inhalation and some intravenous agents (e.g., pentobarbital) at clinical concentrations. The studies presented here utilize a variety of in vivo and in vitro techniques to demonstrate that general anesthetics significantly inhibit several forms of voltage-gated calcium channels. Particular care must be taken in interpreting experiments involving general anesthetics to ensure their clinical applicability, as unrealistically high concentrations can lead to false conclusions. While ligand-gated channels such as GABAA and NMDA receptors have been shown to underlie many anesthetic endpoints, there are still many gaps that voltage-gated calcium channels are able to fill. More research is needed not only on calcium channels, but on other possible related targets of general anesthetics, for a better understanding of the mechanisms behind these ubiquitous and useful agents.

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