Putative Roles of Astrocytes in General Anesthesia

Daniel K. Mulkey1,*, Michelle L. Olsen2, Mengchan Ou3, Colin M. Cleary1 and Guizhi Du3

1Department of Physiology and Neurobiology, University of Connecticut, Storrs CT, USA; 2School of Neuroscience, Virginia Polytechnic and State University, Blacksburg, VA, USA; 3Department of Anesthesiology, West China Hospital, Sichuan University, China

Abstract: General anesthetics are a mainstay of modern medicine, and although much progress has been made towards identifying molecular targets of anesthetics and neural networks contributing to endpoints of general anesthesia, our understanding of how anesthetics work remains unclear. Reducing this knowledge gap is of fundamental importance to prevent unwanted and life-threatening side-effects associated with general anesthesia. General anesthetics are chemically diverse, yet they all have similar behavioral endpoints, and so for decades, research has sought to identify a single underlying mechanism to explain how anesthetics work. However, this effort has given way to the ‘multiple target hypothesis’ as it has become clear that anesthetics target many cellular proteins, including GABA\textsubscript{A} receptors, glutamate receptors, voltage-independent K\textsuperscript{+} channels, and voltage-dependent K\textsuperscript{+}, Ca\textsuperscript{2+} and Na\textsuperscript{+} channels, to name a few. Yet, despite evidence that astrocytes are capable of modulating multiple aspects of neural function and express many anesthetic target proteins, they have been largely ignored as potential targets of anesthesia. The purpose of this brief review is to highlight the effects of anesthetic on astrocyte processes and identify potential roles of astrocytes in behavioral endpoints of anesthesia (hypnosis, amnesia, analgesia, and immobilization).

Keywords: Astrocyte, general anesthesia, mechanism, ion channel, synaptic, neuron-astrocyte communication.

1. INTRODUCTION
1.1. Overview of Astrocyte Physiology

Historically, astrocytes have been relegated to a supportive role, subserving their neuronal counterparts; however, this perspective is rapidly evolving. Astrocytes are now accepted as the third element of tripartite synapse, where they contribute to the development and maintenance of synapses and maintain low ambient levels of the primary excitatory neurotransmitter glutamate. They are significant contributors to GABA uptake and contribute to the maintenance of [K\textsuperscript{+}]\textsubscript{o} [1]; thus, at the most basic level, astrocytes serve to regulate neural and network excitability. Astrocytes also enwrap >99% of the cerebral vasculature, where they contribute to neurovascular coupling [2, 3] and blood-brain barrier function [4]. This cell population is exquisitely sensitive to perturbations in their local environment, as evidenced by the ability to sense a single synaptic event [5]. Astrocytes also typically function as a gap junction coupled syncytium to regulate neural activity in response to various cues, including changes in metabolic state, pH, CO\textsubscript{2}, O\textsubscript{2} redox state, and sleep-wake state [1]. It is also becoming clear that astrocytes show functional heterogeneity within and between brain regions in healthy and diseased brains [6]. The advent of astrocyte-specific molecular targeting strategies, optical probes, and technological advances in imaging has revealed that this functionally heterogeneous cell population contributes to central nervous system function in the micro/nanoscopic compartments of the brain but also serve to regulate complex behaviors, including sleep [7, 8], respiration [9, 10], circadian rhythms [11, 12], plasticity, information processing and cognition [13, 14] (for review see [106]) and potentially brain state [15].

2. ANESTHETIC SENSITIVE ASTROCYTE PROCESSES

2.1. Astrocyte-astrocyte Communication

Astrocytes are highly coupled to one another by the formation of gap junctions at points of contact, thus forming large networks or syncytia of coupled astrocytes [16]. One of the first studies to evaluate the effects of anesthetics on astrocyte function demonstrated that gap junctional coupling between astrocytes in culture can be completely abolished in the presence of 10^{-7} M propofol and etomidate [17]. Further, the volatile anesthetics halothane, enflurane, and isoflurane cause a dose-dependent and reversible inhibition of astrocyte gap junctions, thus leading to astrocyte coupling. A subsequent study demonstrated that propofol (at clinically relevant concentrations of 10^{-5} M - 10^{-4} M) caused an increase in intracellular Ca\textsuperscript{2+} levels sufficient to uncouple astrocyte gap junctions [18]. These early culture studies were followed up using in situ slice preparations where gap junction communication between astrocytes was assessed by dye coupling.
Here, propofol (100 and 150 µM) strongly and reversibly inhibited astroglial network communication as assessed by dye coupling in layer I somatosensory cortical astrocytes. Acute alterations in astrocyte dye coupling were associated with enhanced phosphorylation of connexin 43 (Cx43)- the primary astrocyte gap junction protein [20]. Importantly, phosphorylation changes associated with anesthetic exposure persisted in the presence of tetrodotoxin (TTX; to block neuronal action potentials) and the glutamate receptor antagonist CNQX and APV, indicating a direct effect of propofol on the astrocyte gap junctions [20]. The above studies demonstrate that astrocyte-astrocyte communication is disrupted in the presence of anesthetic and may play a role in the anesthetic mechanism of action.

Intracellular Ca\(^{2+}\) signaling in the form of intrinsic oscillatory, propagating waves, and dynamic changes in Ca\(^{2+}\) in microdomains represent a primary mode of communication between coupled astrocytes in response to neuronal signaling and other changes in the extracellular environment [21]. Recent work demonstrated that isoflurane, ketamine/xyazine as well as urethane suppressed and desynchronized astrocyte Ca\(^{2+}\) signaling in awake, head-restrained mice [22]. For these studies, astrocytes were identified by eGFP expression driven by an astrocyte-specific promoter and bulk labeled with the Ca\(^{2+}\) indicator rhod-2. Using two-photon imaging in the neocortex, Ca\(^{2+}\) signaling was compared in astrocytes between awake animals and those exposed to 30 minutes of anesthesia (1.0-1.5% isoflurane, 0.12 mg·g\(^{-1}\) ketamine combined with 0.01 mg·g\(^{-1}\) xyazine or 1.25 mg·g\(^{-1}\) urethane). Anesthesia also inhibited Ca\(^{2+}\) responses to whisker stimulation typically observed in awake mice. Remarkably, anesthesia induced suppression of astrocyte Ca\(^{2+}\) signaling appeared independent of neuronal activity as blocking neuronal activity with TTX did not affect astrocyte Ca\(^{2+}\) events. Additional work using a genetically encoded astrocyte targeted Ca\(^{2+}\) indicator, GCamp6f, coupled with 3D- two photon Ca\(^{2+}\) imaging, a technical approach sufficient to capture Ca\(^{2+}\) dynamics in astrocyte processes in vivo in response to the firing of a single action potential [23], demonstrated spontaneous Ca\(^{2+}\) activity in astrocytes of the somatosensory cortex which was reduced by nearly 85% under isoflurane anesthesia (2%) when compared to awake mice. A similar result (55% reduction in spontaneous activity) was observed in hippocampal and cortical brain slices using the same imaging paradigm [23]. The above studies provide evidence that anesthesia, at clinically relevant concentrations, attenuates astrocyte Ca\(^{2+}\) signaling, an effect that certainly impacts astrocyte communication within a single cell and astrocyte-astrocyte communication but also likely impacts transmission of astrocyte communication with neighboring neurons, other glial populations, and vascular cells Table 1.

2.2. Regulation of Extracellular Transmitter Content

2.2.1. Glutamate

The clearance of glutamate from the extracellular space and the cessation of glutamatergic transmission occur primarily via the Na\(^+\)-dependent astrocyte glutamate transporters EAAT1 and EAAT2 (Glast and Glut-1 in rodents, respectively). Glutamate transporters maintain low ambient levels of glutamate (<1 µM), ensuring a high signal-to-noise ratio, shape synaptic currents, and prevent chronic activation of glutamate receptors and spillover at glutamatergic synapses [24, 25]. Early studies in cultured astrocytes indicated that glutamate uptake was augmented in a dose (1-3%) and time (0-35 minutes) dependent manner in the presence of isoflurane. This effect was abrogated when Na\(^+\) was omitted from the bathing solution, indicating a reliance on external Na\(^+\); however, this effect was also inhibited using a glutamate transport inhibitor 1-trans-pyrroline-2,4-dicarboxylate (1-trans-PDC) [26]. A similar increase in glutamate uptake was observed in the presence of other volatile anesthetics, including halothane (1-2%) [27]. The near-immediate results observed in these studies suggest that volatile anesthetics may affect turnover time or trafficking of glutamate transporters at the astrocyte plasma membrane. While these studies provide evidence that volatile anesthetics affect glutamate transport in astrocyte cultures, no follow-up studies evaluating astrocyte glutamate transport in situ have been performed. The intravenous anesthetics ketamine and pentoobar-

| Table 1. Effects of Anesthetics on astrocyte processes. |
|--------------------------------------------------------|
| **Astrocyte-Astrocyte Communication**                  |
| Isoflurane                                             |
| inhibits gap junction coupling\(^a\)                     |
| decreases Ca\(^{2+}\) spikes\(^b\)                      |
| Halothane                                             |
| inhibit gap junction coupling\(^a\)                     |
| Propofol                                              |
| inhibit gap junction coupling\(^a\)                     |
| **Regulation of Extracellular Transmitters**            |
| Isoflurane                                             |
| increased glutamate uptake in cell culture\(^c\),       |
| increase GABA release for tonic inhibition\(^d\),       |
| decrease GABA transporter activity\(^e\)                |
| Halothane                                             |
| increased glutamate uptake in cell culture\(^c\),       |
| increase GABA transporter activity\(^f\)                |
| Propofol                                              |
| increase [GABA], and tonic inhibition\(^g\)             |
| **Astrocyte Membrane Properties**                      |
| Isoflurane                                             |
| activates Kir4.1/5.1 channels\(^i\)                     |
| Morphology                                             |
| reduced GFAP and alpha-tubulin\(^i\)                   |
| Metabolic Support                                      |
| reduced lactate release\(^j\)                          |
| **Regulation of Cerebrovascular Tone**                 |
| Isoflurane                                             |
| vasodilation\(^k\), decrease neurovascular coupling\(^n\)|
| Halothane                                             |
| vasodilation\(^m\)                                     |
| Propofol                                              |
| vasodilation\(^n\)                                     |

\(^{18}\), \(^{22}\), \(^{26}\), \(^{31}\), \(^{32}\), \(^{33}\), \(^{77}\), \(^{16}\), \(^{37}\), \(^{48}\), \(^{55}\), \(^{60}\), \(^{65}\), \(^{66}\).
bital demonstrated no immediate effect on glutamate transport [27].

2.2.2. GABA

Diffusion of GABA from the synapse can activate extrasynaptic GABA_A receptors and mediate a potent form of inhibition called tonic inhibition. Extracellular GABA is regulated by members of the solute carrier 6 (SLC6) transporter family (GAT1-4) that transport GABA typically in conjunction with Na^+ and Cl^− [28]. These transporters are differentially expressed by neurons and astrocytes, and physiological disruption of GAT function has been shown to increase tonic inhibition and suppress neural activity [29]. Anesthetics also increase tonic inhibition. For example, propofol and isoflurane [30, 31] directly activate extrasynaptic GABA_A receptors to increase tonic inhibition. However, evidence from reduced preparations including a heterologous cell expression system [32] and synaptosomes [33] also suggests that volatile anesthetics can inhibit GABA transport.

3. ASTROCYTE MEMBRANE PROPERTIES

Electrically, astrocytes are non-excitable cells. Mature astrocytes in situ, across brain regions, are characterized by a large leak K^+ conductance, hyperpolarized resting membrane potentials, and low input resistance <20 MOhms [34]. These properties are mediated in large part by the expression of a repertoire of K^+ channels, with a recent focus on the two-pore channel Trek-1 (gene name Kcnk2) and the inwardly rectifying potassium channel Kir4.1 (Kcnj10).

3.1. Anesthetic Sensitive K^+ Channels

Trek-1 is a two-pore potassium channel (K2P) highly expressed in astrocytes, neurons, and glial cells, including astrocytes, throughout the CNS [35]. Roles of this channel include facilitation of K^+ diffusion and stabilization of the resting membrane potential in response to lipid binding, acidification of the intracellular pH, mechanosensitive stimuli, and heat [36]. Trek-1 is activated by isoflurane and other volatile anesthetics at clinically relevant doses, a response that is dependent on the intracellular C-terminus of the channel [37]; channel activation involves lipid binding to this portion of the channel following administration of anesthetic [38]. Trek-1 null mice show decreased sensitivity to volatile anesthetics, including isoflurane, chloroform, halothane, sevoflurane, and desflurane [39]. Trek-1 activation by anesthetics can hyperpolarize resting membrane potential and suppress firing [40], potentially contributing to the acute effects of anesthesia. As indicated above, Trek-1 is expressed in astrocytes and contributes to astrocyte passive K^+ conductance, linear current-voltage relationship [41], and the hyperpolarized resting membrane potential [42] characteristics of mature astrocytes. Because astrocyte resting membrane potential is near the reversal potential of K^+, it is unlikely that activation of Trek-1 will facilitate further hyperpolarization. However, Trek-1 channels can also serve as a conduit for glutamate release in response to activation of Gβγ signaling [43], so it is possible that anesthetic activation of Trek-1 disrupts this mode of gliotransmission.

Kir4.1 is an inwardly rectifying potassium channel most highly expressed in astrocytes but with significant expression in other glial cell populations [35]. Kir4.1 is developmentally upregulated in astrocytes and, like Trek-1, contributes to the biophysical membrane properties of astrocytes. Kir4.1 has a high open probability at rest and thus contributes to the hyperpolarized resting membrane potential, large K^+ leak conductance, and low input resistance observed in astrocytes [44]. Importantly, this channel is also implicated in extracellular potassium (K^+o) uptake following neuronal activity by a process termed K^+ buffering. Potassium buffering involves the uptake of K^+ via channel-mediated and transporter mechanisms and redistribution of these K^+ ions through a network of coupled neighboring astrocytes and subsequent release in regions of lower [K^+o] [44]. Kir4.1 is a weakly rectifying channel and is thus well-suited to participate in K^+ uptake and release as dictated by the electrochemical driving force for K^+. Kir4.1 exists as a homomer but also forms heteromeric channels with Kir5.1 subunits to form a pH-sensitive K^+ channel [45] with the highest expression found in brainstem regions [46]. In contrast to activation by anesthetics as seen with Trek-1, we have recently demonstrated that pH-sensitive Kir4.1.5.1 heteromeric channels in astrocytes of the retrotrapezoid nucleus, a brainstem region involved in respiratory control [47], were inhibited by isoflurane at clinically relevant concentrations (1% and 3%), a result that was recapitulated in a heterologous expression system (HEK cells) [48]. Conversely, isoflurane had negligible effects on Kir4.1 homomeric channels [48]. Anesthetic inhibition of Kir4.1.5.1 channels is expected to diminish K^+ buffering capacity, depending on factors like size of the extracellular space and compensatory contributions by anesthetic activated Trek-1, this may increase extracellular K^+ and favor increased neural activity. In this regard, it is worth noting that Kir4.1 and Kir5.1 transcripts are expressed at higher levels in the brainstem compared to the cortex [46], which may contribute to differential anesthetic sensitivities between these regions.

3.2. Astrocyte Morphology

Mature astrocytes are perhaps the most morphologically complex cells in the mammalian brain. In the adult CNS, these cells occupy non-overlapping domains with estimates indicating a single, mature rodent astrocyte encompassing between 20,000 - 80,000 μm^2 of domain space [49, 50], associated with 300-600 neuronal dendrite [50], and contacting more than 100,000 individual synapses [49], numbers that increase by 30-folds in the human brain [51]. This complex morphology is achieved, in part, by changes in the activation state of the small RhoGTPases (Rho, Rac1, and ROCK) which mediate cytoskeletal actin dynamics [52]. In C6 glioma cells, a commonly used model for glial cells, stellation is largely reversed in the presence of clinically relevant (1%) concentrations of the volatile anesthetic isoflurane and beta-receptor agonist isoproterenol. This effect can be completely blocked with the application of two specific Rho-kinase inhibitors (Y-27632 and HA1077) [53]. Subsequent studies revealed that long-term exposure to high levels of isoflurane (3%) was toxic to immature astrocytes in culture, inducing a significant decrease in expression of the small RhoGTPase, RhoA, and major alterations in the astrocyte actin cytoskele-
ton [54]. Further studies using lower concentrations of isoflurane (1.4%) for four hours demonstrated no effect on F-actin but reduced expression of two astrocyte filament proteins, alpha-tubulin and glial fibrillary acidic protein (GFAP) [55]. These and other studies suggest that anesthetics influence astrocyte morphology, but it is unclear at this point if anesthetics mediate these changes in vivo on an acute time scale or if changes in astrocyte morphology contribute to the effects of anesthesia.

3.3. Metabolic Support

Glucose is the universal substrate for energy production in the CNS. During normal levels of activity, glucose is metabolized in astrocytes and neurons through glycolysis to generate ATP. Astrocytes, rather than neurons, have the ability to store excess glucose as glycogen, an energy reserve found in astrocyte end-feet surrounding brain vasculature and in the perisynaptic process [56, 57]. During periods of high metabolic activity, glycogen is broken down into pyruvate and L-lactate in a process called glycogenolysis, resulting in ATP generation. The astrocyte-neuron lactate shuttle hypothesis posits that astrocytes, localized with processes in direct contact with blood vessels, take up glucose from the vasculature and convert excess glucose to glycogen. During periods of high metabolic demand, glycogen is broken down to L-lactate and shunted to neurons to fuel neuronal oxidative phosphorylation [58]. Brain lactate concentrations are ~30% higher during wakefulness than during sleep, or ketamine/xylazine induced anesthesia, as assessed by microdialysis [59], a result that was attributed to enhanced glycophytic clearance. However, a more recent study measuring lactate release demonstrated a 5-10% reduction in lactate M) in the release in response to thiopental, propofol, and etomidate (each at 10 acute brainstem and cortical slices [60]. Both thiopental and etomidate caused reductions in the cytosolic [NADH] /[NAD+] ratio in cultured astrocytes, similar to that induced by glucose deprivation. Further, these authors demonstrated a near 25% decrease in brain lactate in acute cortical slices harvested during the wake (light) phase compared to the dark phase. This work suggests that anesthetics may directly interfere with astrocyte lactate production or release.

4. REGULATION OF CEREBROVASCULAR TONE

Astrocytes can modify cerebrovascular tone to increase blood flow in response to neural activation (neurovascular coupling) [3, 61] or high CO2/H+ (CO2/H+ vascular reactivity) [62-64] by several mechanisms involving Ca2+ signaling and release of vasoactive signals (nitric oxide, purines, or arachidonic acid and related metabolites). Although anesthetics typically increase cerebral blood flow [65] and decrease neurovascular coupling [66], these are mediated largely by diminished neural activity or direct effects on vascular endothelial cells and smooth muscle. However, since certain anesthetics (isoflurane and urethane) have been shown to suppress sensory-evoked Ca2+ responses in astrocytes [22], it is possible that astrocyte-dependent regulation of blood flow in response to neural activity is also compromised under these conditions.

5. POTENTIAL CONTRIBUTIONS OF ASTROCYTES TO ANESTHETIC ENDPOINTS

Astrocytes contribute to behaviors that are affected by anesthetics under normal conditions and express many proteins targeted by anesthetics, including GABA_A receptors [67], NMDA receptors [68], background K+ channels [41], and inward rectifying K+ [48]. Therefore, highlighted below are potential mechanisms by which astrocytes contribute to anesthetic endpoints.

6. UNCONSCIOUSNESS

General anesthetics are able to induce a state of unconsciousness, characterized by a loss of perception or responsiveness to environmental stimuli, at concentrations much lower than required for immobilization [69, 70]. In rodents, loss of the righting reflex - the ability to stand on all fours after being placed in the supine position - is commonly used as a surrogate for unconsciousness [71]. Neural circuits responsible for consciousness are not well understood, and consequently, the same is true for anesthetic-induced unconsciousness. Studies have shown that many anesthetics activate sleep-promoting neurons, including those in the ventrolateral preoptic region, tuberomammillary region, and brainstem pontine reticular formation. Simultaneously, anesthetics can inhibit arousal-promoting monoaminergic systems and cholinergic neurons in the pedunculopontine and laterodorsal tegmental areas, histaminergic neurons in the tuberomammillary nucleus, adrenergic locus coeruleus neurons, serotonergic dorsal raphe neurons, and others. At the systems level, anesthetics like propofol have also been shown to disrupt cortical and thalamocortical connectivity [72, 73], suggesting disruption of synaptic communication contributes to anesthetic-induced unconsciousness [70, 74, 75].

As noted above, astrocytes are integral components of the synapse [76], show Ca2+ responses to wake-on neurotransmitters [77], and are thought to contribute to consciousness [78-80]. The studies mentioned above indicate that anesthetics directly impact astrocyte protein function and suppress Ca2+ signaling by a mechanism independent of neural activity; therefore, it is possible that astrocytes contribute to anesthetic-induced loss of consciousness. Consistent with this, ketamine has been shown to inhibit spontaneous glutamate release from astrocytes leading to suppression of slow inward currents (SICs) recorded in cortical pyramidal neurons [81]. Considering SICs promote synchronized neural activity [82] and that these are fundamentally important for cognition [83], suppression of this astrocyte-dependent process may contribute to anesthetic-induced connectivity problems and loss of consciousness. Interestingly, opioids, which are commonly used in conjunction with general anesthetics to reduce pain [75], have the opposite effect on glutamate signaling from hippocampal astrocytes. For example, hippocampal astrocytes express µ-opioid receptors [84] and, when activated, release glutamate through a background K+ channel (Trek-1) [85] to facilitate LTP and memory performance [84]. However, the extent to which this mechanism contributes to anesthesia remains unclear.
7. AMNESIA

The hypnotic effects of anesthetics also involve loss of memory (amnesia), and this endpoint occurs at anesthetic levels much lower than those required for unconsciousness or immobility [30, 74]. For example, in humans and other animals, the steady-state concentration of isoflurane that impairs auditory, verbal, or visual memory is ~25% of the minimum alveolar concentration (MAC) that prevents movement in response to a painful stimulus in 50% of subjects [86] (MAC is a common index of anesthetic potency; [87]). The hippocampus and frontal cortex are essential for learning and memory [88, 89], and low concentrations of various anesthetics have been shown to suppress hippocampal long-term potentiation (LTP) [90-93], a form of synaptic plasticity considered to be a cellular correlate of memory [94]. Evidence suggests that astrocytes are necessary for synaptic plasticity [14], and targeted chemogenetic activation of astrocytes is sufficient to induce LTP in the hippocampus and improved memory performance [95], whereas chemogenetic inhibition of hippocampal astrocytes impaired memory performance in mice [96].

Mechanisms by which astrocytes contribute to learning and memory include the release of the purines, that favor suppression of synaptic activity [97], D-serine, which functions as an NMDA receptor agonist required for LTP [14], and lactate, which is required for maintenance of LTP [98]. Note that this list is not all inclusive, and other gliotransmitters are also thought to contribute to memory [99]. Vesicular release of neuromodulators from astrocytes has been shown to contribute to memory function [100]; however, many of these modulators are also released from astrocytes through connexin hemichannels [101-103], and disruption of this release pathway is associated with memory problems. For example, blocking connexin hemichannels and gap junctions (specifically Cx43, the main connexin in astrocytes) during memory consolidation caused amnesia for auditory fear conditioning (a form of long-term memory) in rats, and this deficit could be rescued by the application of a gliotransmitter cocktail (glutamate, glutamine, lactate, d-serine, glycine, and ATP) [104]. Connexin hemichannels are also blocked by various general anesthetics [19, 105], and so may be a mechanism by which astrocytes contribute to anesthetic-induced amnesia.

Anesthetic suppression of metabolic activity likely contributes to cognitive impairment, particularly during emergence from anesthesia. For example, mitochondrial complex I is inhibited by anesthetics [106] and astrocyte-specific Ndufs4 (a gene that encodes a subunit of complex I) knockout mice showed increased anesthetic (isoflurane and halothane) sensitivity as measured using the righting reflex (hypnotic) or tail clamp (pain and immobilization) but only when emerging from the anesthetic state (i.e., recovered their response at lower anesthetic concentrations compared to control mice). Although it is tempting to speculate this mechanism involves limited astrocyte metabolic capacity, others have shown that volatile anesthetics actually increase mitochondrial ATP production even in Ndufs4 null mice by augmenting the activity of other components of the mitochondrial respiratory chain [107]. Furthermore, volatile anesthetics have been shown to increase brain glycogen levels in a manner that correlates with the depth and duration of anesthesia. For example, pharmacological inhibition of glycogen phosphorylase, an enzyme preferentially expressed by astrocytes that mediates glycogen breakdown, increased glycogen levels in the cortex, hippocampus, and thalamus, and prolonged recovery of the righting reflex during emergence from isoflurane anesthesia [108]. Conversely, increasing glycogen phosphorylase activity conditionally in astrocytes decreased brain glycogen levels and decreased isoflurane sensitivity as evidenced by a delayed time of loss of the righting reflex (induction) and shortened time to recovery (emergence) of the righting reflex [108]. Based on these results, emergence from anesthesia appears to be particularly sensitive to astrocyte metabolism. However, anesthetics also suppress astrocyte glycogen and lactate release [60], and as noted above, lactate is essential for LTP and long-term memory formation [98, 109]. Therefore, this may be another mechanism by which astrocytes contribute to anesthetic-induced amnesia.

Anesthetic-induced potentiation of inhibitory GABA signaling also contributes to the hypnotic effects of anesthetics. Astrocytes express high levels of two GABA transporters (GAT1 and GAT3) and so are important for regulating extracellular GABA content, and consequently, the level of tonic inhibition [110]. Anesthetics have been shown to increase tonic inhibition in the hippocampus partly by direct anesthetic activation of neural extrasynaptic α5 subunit-containing GABA_A receptors [30]. However, other contributing factors may include anesthetic suppression of GABA clearance [32, 33] and increased expression of GABA_A receptors [111].

8. ANALGESIA

Acute pain is triggered by activation of nociceptive nerve terminals (cell bodies located in the dorsal root ganglia) that relay this signal to neurons in the dorsal horn of the spinal cord followed by supraspinal neurons in the brainstem, thalamus (ventral posterior nucleus, the intralaminar nucleus and the parafascicular nucleus), and cortex where the pain is perceived [112]. Astrocytes may limit pain under normal conditions by releasing purines or anti-inflammatory cytokines like type-I interferons [113]. However, tissue damage or inflammation can cause the pain pathway to become hypersensitive, and astrocytes appear to contribute to this sensitization. For example, administration of fluoroacetate (a metabolic poison preferentially taken up by astrocytes that at low doses is thought to inhibit astrocyte function) blunted pain associated with chronic inflammation as well as nerve injury [114]. The facilitatory role of astrocytes in chronic pain involves the release of various inflammatory factors (e.g., interleukin 1β, tumour-necrosis factor-α, prostaglandin E2) as well as dysregulation of glutamate uptake [112, 113]. Many of these paracrine signals are released from astrocytes via connexin hemichannels, and astrocyte expression of connexins (e.g., Cx43) has been shown to increase following nerve injury [113], potentially increasing the contribution of astrocytes to chronic pain. Consistent with this, pharmacological [115, 116] and genetic [117] disruption of connexin hemichannels and gap junctions diminished neuropathic pain.
in mice. Considering certain anesthetics also inhibit connexins [17, 19], a similar mechanism may also contribute to anesthetic-induced analgesia.

Chronic pain may also result from diminished K\(^+\) channel expression that can contribute to neural hyperexcitability directly [118] and indirectly by reduced K\(^+\) uptake by astrocytes or astrocyte-like satellite cells in the DRG [119]. Consistent with this possibility, genetic silencing of Kir4.1 expression in DRG satellite cells by injection of a Kir4.1 dominant-negative construct caused a pain phenotype in the absence of injury [119]. Potassium channels, including some expressed by astrocytes, are activated by anesthetics, and to the extent that extracellular K\(^+\) accumulation contributes to chronic pain, anesthetic activation of astrocyte K\(^+\) channels should facilitate K\(^+\) uptake and contribute to analgesia. For example, volatile but not intravenous anesthetics activate several background K\(^+\) channels [120], including Trek-1 that is highly expressed by astrocytes [41]. However, the relevance of Trek-1 in astrocyte physiology remains unclear since the genetic ablation of this channel minimally affected the passive electrical properties of astrocytes [121].

9. IMMOBILIZATION

Immobility - lack of purposeful movements - is mediated primarily at the level of the spinal cord where volatile anesthetics (e.g., isoflurane) target ventral spinal interneurons and motoneurons more than dorsal horn neurons involved in nociception [122, 123]. Hence, anesthetics can differentially affect pain and motor pathways. Furthermore, spinal transection can reduce the MAC response to a painful stimulus by ~50% [124], suggesting descending locomotor drive from mesencephalic locomotor regions (e.g., the cuneiform and pedunculopontine nuclei; [125]) counteracting anesthetic-induced depression at the spinal cord level through descending facilitation [126].

10. Motor behavior requires the coordinated activity of motor neurons as well as premotor networks. Although the potential roles of astrocytes in motor behavior are not well understood, astrocytes in the brainstem [127] and spinal cord [128] premotor networks have been shown to respond to sensory input and communicate with neurons in a manner that influences neural responses. The ability of astrocytes to form a gap junction coupled syncytium allows them to regulate a broad population of neurons simultaneously and thus coordinate downstream motor neurons. As noted above, anesthetics block connexin hemichannels and gap junctions [19, 105], which conceivably will disrupt gliotransmitter release and prevent coordination between astrocytes.

10. CARDIORESPIRATORY FUNCTION IS PRESERVED UNDER GENERAL ANESTHESIA

A remarkable feature of general anesthesia is that autonomic and respiratory systems remain operational even at immobilizing anesthetic concentrations. At least part of the mechanism responsible for maintaining cardiorespiratory output under these conditions involves functionally distinct populations of neurons located in the rostral ventrolateral medulla (RVLM); pre-sympathetic C1 neurons that regulate blood pressure and respiratory chemoreceptors that regulate breathing in response to changes in CO\(_2\)/H\(^+\). For example, C1 neurons appear more active in rats anesthetized with volatile anesthetics, and archaerhodopsin-mediated inhibition of C1 neurons caused a larger drop in blood pressure in isoflurane-anesthetized rats compared to unanesthetized controls [129]. Recent evidence also suggests that RVLM astrocytes function as baroreceptors by sensing decreases in cerebral perfusion pressure and responding by Ca\(^{2+}\)-dependent vesicular release of ATP to activate blood pressure regulating RVLM neurons and sympathetic output [130]. Several anesthetics decrease cerebral blood flow [65] and so may engage this compensatory mechanism. However, this mechanism involves activation of mechanosensitive TRPV4 channels and ATP release through Cx43 hemichannels [131], and since propofol inhibits TRPV4 [132] and propofol and volatile anesthetics can block Cx43 [17, 20], we suspect this mechanism minimally contributes to the maintenance of blood pressure during exposure to these drugs. Furthermore, unlike most levels of the respiratory circuit, volatile anesthetics activate chemosensitive neurons in a region of the RVLM called the retrotrapezoid nucleus (RTN) by mechanisms involving inhibition of Thik-1 background K\(^+\) channels [133] and activation of a leak Na\(^+\) conductance [134]. This mechanism likely helps in maintaining the respiratory drive during general anesthesia. Interestingly, recent evidence suggests that heteromeric Kir4.1/5.1 channels in astrocytes located in the RTN and near C1 neurons are also inhibited by isoflurane [48]. These channels are expressed at higher levels by astrocytes in the brainstem compared to the cortex [46, 135], and since they are the main conduit for K\(^+\) uptake by astrocytes, inhibition by isoflurane is expected to increase extracellular K\(^+\) to activate RTN chemoreceptors and C1 neurons to help preserve cardiorespiratory function when other elements of the central nervous system are depressed.

CONCLUSION

Astrocytes contribute to anesthetic sensitive behaviors, express many proteins targeted by anesthetics, and anesthetics perturb several key astrocyte processes. Therefore, astrocytes likely contribute to features of general anesthesia. However, the mechanisms and extent to which they contribute remain unclear. Nevertheless, the work summarized here identifies astrocytes as common denominators for otherwise disparate anesthetic processes (induction and emergence) and endpoints. This work also highlights the need to use genetic targeting strategies to manipulate distinct processes in astrocytes that are anesthetic sensitive in an effort to further increase our understanding of the effects of anesthetics on the CNS.

LIST OF ABBREVIATIONS

| Abbreviation | Description                     |
|--------------|---------------------------------|
| [K\(^+\)]\(_{o}\) | Extracellular potassium          |
| APV          | 2-Amino-5-phosphonovalerate     |
| CNQX         | 6-cyano-7-nitroquininaline-2,3-dioneis |
| CO\(_2\)/H\(^+\) | Hypercapnic acidosis            |
| Cx43         | Connexin 43                     |
| DRG          | Dorsal root ganglion            |
EAAT = Excitatory amino acid transporter

eGFP = Enhanced green fluorescent protein

GABA<sub>A</sub> = Gamma-aminobutyric acid A receptor

GAT = Gamma-Aminobutyric acid transporter

Kir4.1 = Inward rectifying K+ channel 4.1

LTP = Long term potentiation

I-trans-PDC = I-trans-pyridoline-2,4-dicarboxylate

NMDA = N-methyl-D-aspartate receptor

Redox = Reduction oxidation

RVLM = Rostral ventrolateral medulla

SICs = Slow inward currents

SLC6 = Solute carrier 6

THIK-1 = Two-pore domain halothane inhibited K+ channel

Trex-1 = TWIK-related K+ channel

TRPV4 = Transient Receptor Potential Cation Channel Subfamily V Member 4

TTX = Tetrodotoxin

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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