Cellular stress and AMPK links metformin and diverse compounds with accelerated emergence from anesthesia and potential recovery from disorders of consciousness

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ABSTRACT

The neural correlates of consciousness and the mechanisms by which general anesthesia (GA) modulate such correlates to induce loss of consciousness (LOC) has been described as one of the biggest mysteries of modern medicine. Several cellular targets and neural circuits have been identified that play a critical role in LOC induced by GA, including the GABA receptor and ascending arousal nuclei located in the basal forebrain, hypothalamus, and brain stem. General anesthetics (GAs) including propofol and inhalational agents induce LOC in part by potentiating chloride influx through the GABA receptor, leading to neural inhibition and LOC. Interestingly, nearly all GAs used clinically may also induce paradoxical excitation, a phenomenon in which GAs promote neuronal excitation at low doses before inducing unconsciousness. Additionally, emergence from GA, a passive process that occurs after anesthetic removal, is associated with lower anesthetic concentrations in the brain compared to doses associated with induction of GA. AMPK, an evolutionarily conserved kinase activated by cellular stress (e.g. increases in calcium [Ca2+] and/or reactive oxygen species [ROS], etc.) increases lifespan and healthspan in several model organisms. AMPK is located throughout the mammalian brain, including in neurons of the thalamus, hypothalamus, and striatum as well as in pyramidal neurons in the hippocampus and cortex. Increases in ROS and Ca2+ play critical roles in neuronal excitation and glutamate, the primary excitatory neurotransmitter in the human brain, activates AMPK in cortical neurons. Nearly every neurotransmitter released from ascending arousal circuits that promote wakefulness, arousal, and consciousness activates AMPK, including acetylcholine, histamine, orexin-A, dopamine, and norepinephrine. Several GAs that are commonly used to induce LOC in human patients also activate AMPK (e.g. propofol, sevoflurane, isoflurane, dexmedetomidine, ketamine, midazolam). Various compounds that accelerate emergence from anesthesia, thus mitigating problematic effects associated with delayed emergence such as delirium, also activate AMPK (e.g. nicotine, caffeine, forskolin, carbachol). GAs and neurotransmitters also act as preconditioning agents and the GABA receptor inhibitor bicuculline, which reverses propofol anesthesia, also activates AMPK in cortical neurons. We propose the novel hypothesis that cellular stress-induced AMPK activation links wakefulness, arousal, and consciousness with paradoxical excitation and accelerated emergence from anesthesia. Because AMPK activators including metformin and nicotine promote proliferation and differentiation of neural stem cells located in the subventricular zone and the dentate gyrus, AMPK activation may also enhance brain repair and promote potential recovery from disorders of consciousness (i.e. minimally conscious state, vegetative state, coma).

Introduction

One of the greatest and most profound mysteries in all of modern medicine is identification of the neural correlates that underlie human consciousness and the mechanisms through which general anesthesia (GA) induce a loss of consciousness (LOC) [1]. The use of general anesthetics to successfully induce LOC and maintain GA in millions of patients each year provides a unique opportunity to assess and determine if specific neural circuits play critical roles in promoting consciousness [2]. Consciousness is characterized by wakefulness, arousal, cognition, self-awareness, and awareness of one’s environment and LOC may be easily assessed by a patient’s lack of response to a verbal command from a clinician [3]. Loss of the righting reflex (LORR) in rodent models in response to general anesthetics has also proven to be
an excellent surrogate for LOC in human patients, rendering animal models as powerful investigative tools to study consciousness [3,4].

GA, which has been described as a drug-induced reversible coma, is characterized by unconsciousness, amnesia, akinesia (loss of voluntary movement), and analgesia (loss of response to pain) and typically consists of three periods [3]. During the initial period, known as induction, a bolus dose of an anesthetic drug is administered that leads to LOC as evidenced by a lack of response to an oral command. However, an intriguing phenomenon known as paradoxical excitation may also occur after initial administration of an anesthetic drug [3-5]. When administered at a low dose, nearly every anesthetic induces behavioral signs of neuronal activation such as eccentric body movements and a transient increase in beta activity (13–25 Hz) on the electroencephalogram (EEG) [3,5]. Consequently, many anesthetics appear to paradoxically excite the brain before inducing unconsciousness [3,6].

After induction and LOC, the maintenance period of GA is achieved via a combination of drugs and anesthetics and is associated with a decrease in EEG beta activity (phase 1), an increase and anteriorization of lower frequency alpha (8–12 Hz) and delta (0–4 Hz) activity (phase 2), an EEG activity comprising flat periods interspersed with beta and alpha activity (i.e. burst suppression, phase 3), and an isoelectric EEG (i.e. flat, phase 4) [3,7]. Emergence from GA is a passive process that occurs after the removal of anesthesia, approximates an EEG reversal from maintenance period phases 2 or 3, and is characterized by the return of various physiological processes and responses to verbal commands [3,7,8].

Although a number of structurally diverse compounds are used during GA to induce LOC, the GABAA receptor has been identified as a primary target for several anesthetic drugs, including propofol, sevoflurane, isoflurane, and midazolam [9]. By binding post-synthetically to GABAA receptors located on excitatory pyramidal neurons in the brain (e.g. cortex), anesthetics potentiate GABA-mediated increases in chloride (Cl-) influx through the GABAA receptor, leading to neural inhibition and LOC [3,9]. GABA is the primary inhibitory neurotransmitter within the brain and GABA release from cortical interneurons play an important role in inhibition of excitatory pyramidal neurons and LOC [3,7,9]. The NMDA receptor (NMDAR) located on inhibitory interneurons in the cortex, hippocampus, and limbic system has also been shown to be a primary target for ketamine, an anesthetic drug that functions as an NMDAR antagonist and induces LOC by promoting an increase in uncoordinated neural activity [3,7,9]. An additional cellular target contributing to LOC is the α2-adrenergic receptor found on neurons from the locus ceruleus [10]. α2-adrenergic receptor agonists, including dexmedetomidine, clonidine, and xylazine, promote LOC by inhibiting release of norepinephrine in the ventrolateral preoptic nucleus (VLPO) of the hypothalamus [3,10]. As norepinephrine inhibits GABA and galanin release from VLPO neurons, α2 agonist-mediated disinhibition of VLPO neurons contribute to LOC via GABA- and galanin-mediated inhibition of various arousal nuclei in the brain [3,10]. Such nuclei include cholinergic neurons in the lateral dorsal and pedunculopontine tegmental nuclei, histaminergic neurons in the tuberomammillary nucleus, dopaminergic neurons in the ventral periaqueductal gray area, and serotonergic neurons in the dorsal raphe nucleus [3,4,7]. Other arousal nuclei that play important roles in LOC include cholinergic neurons in the basal forebrain, orexinergic neurons in the lateral hypothalamus, dopaminergic neurons in the ventral tegmental area, and glutamatergic neurons in the parabrachial nucleus [3,4,7,11].

AMPK, known as the master regulator of cellular metabolism, is an evolutionarily conserved serine/threonine kinase that increases lifespan and/or healthspan in several model organisms [12,13]. The induction of cellular stress, mediated by increases in the AMP/ADP/ATP ratio, increases in intracellular reactive oxygen species (ROS), or an increase in intracellular calcium (Ca2+) levels leads to AMPK activation via phosphorylation of the AMPK catalytic α subunit by the upstream kinases liver kinase B1 (LKB1) or Ca2+/calmodulin-dependent protein kinase kinase-β (CaMKKβ) or CaMKK2 [12–14]. AMPK activation promotes several cellular processes critical for neuronal function and survival, including mitochondrial oxidative phosphorylation, autophagy, and mitochondrial biogenesis [12,13,15]. Activation of AMPK has also been demonstrated to play a beneficial role in diverse neurodegenerative disorders including Alzheimer’s, Parkinson’s, and Huntington’s disease [16–18]. Numerous structurally dissimilar compounds induce AMPK activation, including the AMP analog AICAR, the anti-diabetic drug metformin, and several plant-derived and naturally-occurring compounds (e.g. caffeine, forskolin, nicotine, etc.) [19–22].

Hypothesis

We propose the novel hypothesis that cellular stress-induced AMPK activation, mediated by increases in intracellular Ca2+, ROS, and/or an AMP(ADP)/ATP ratio increase, etc. links wakefulness, arousal, and consciousness with paradoxical excitation and accelerated emergence from anesthesia. AMPK is located throughout the brain and in areas that are critical for LOC induced by GA, including neurons of the striatum, thalamus, and hypothalamus as well as in pyramidal neurons located in the hippocampus and cortex. Increases in intracellular Ca2+ and ROS activate AMPK and play important roles in facilitating neuronal excitation and glutamate, the primary excitatory neurotransmitter in the human brain, activates AMPK in cortical neurons. Nearly every neurotransmitter released from ascending arousal nuclei that promote wakefulness, arousal, self-awareness, and consciousness activates AMPK, including acetylcholine, histamine, orexin-A, dopamine, and norepinephrine. Several general anesthetics that are commonly used to promote LOC in human patients also induce paradoxical excitation in low doses and activate AMPK (e.g. propofol, sevoflurane, isoflurane, dexmedetomidine, ketamine, and midazolam). Because lower concentrations of anesthetics are present in the brain during emergence from GA compared to the initial induction phase, it is likely that cellular stress-induced AMPK activation in neurons by excitatory neurotransmitters and low doses of anesthetics represents a common mechanism linking paradoxical excitation with return of consciousness and emergence from GA. Indeed, the GABAA receptor inhibitor bicuculline, which reverses propofol anesthesia, also activates AMPK in cortical neurons. Transient increases in ROS, Ca2+, and AMPK and various neurotransmitters and general anesthetics also act as pre-conditioning agents, indicating that preconditioning is analogous to paradoxical excitation and is mediated through a common mechanism of AMPK activation. Furthermore, compounds including nicotine, caffeine, forskolin, and carbamazepine that have recently been shown to accelerate emergence from anesthesia in both animal models and in humans activate AMPK, indicating that restoration of consciousness by structurally distinct compounds may act via AMPK. Moreover, nicotine and the AMPK activator metformin promote proliferation and differentiation of neural stem cells located in the dentate gyrus and the subventricular zone, suggesting that AMPK may also enhance brain repair and promote potential recovery from disorders of consciousness (i.e. minimally conscious state, vegetative state, coma).

Evaluation of the hypothesis

AMPK is present in neurons throughout the brain and is activated by stress and arousal-promoting neurotransmitters.

Wakefulness, arousal, cognition, self-awareness, and awareness of one’s environment are critical components of consciousness and likely require coordinated communication among neural circuits located throughout the brain. As such, any candidate proteins that are hypothesized to play a central role in restoring and/or maintaining consciousness must also possess a ubiquitous presence in the brain. Culmsee et al. found that the AMPK catalytic α2 subunit was present throughout the adult rat brain, including in neurons of the thalamus,
hypothalamus, and striatum as well as in pyramidal neurons in the hippocampus and cortex [23]. The AMPK activator AICAR protected hippocampal neurons from cell death induced by hypoxia and glucose deprivation whereas antisense oligonucleotide-mediated suppression of AMPK α1 and α2 subunits exacerbated cell death following glucose deprivation and abrogated AICAR-mediated neuronal protection [23]. Pyramidal neurons in the cortex are also primary targets of several anesthetic drugs and the thalamus, striatum, and arousal nuclei in the hypothalamus play critical roles in maintaining consciousness, highlighting the potential importance of AMPK in LOC and emergence from GA [3]. As excitatory cortical pyramidal neurons likely represent key components of the neural correlates of consciousness, pyramidal neurons located in the CA1 region of the hippocampus have also been shown to play a critical role in long-term potentiation (LTP), a form of synaptic plasticity that is widely considered to be the cellular correlate of learning and memory [3,24].

The induction of cellular stress is essential in facilitating hippocampal LTP and AMPK is activated by compounds and methodologies that promote LTP [24]. Similar to activation of excitatory pyramidal neurons in the cortex, induction of hippocampal CA1 LTP is dependent on an increase in the levels of intracellular Ca2+ [25]. Ca2+ and ROS have been shown to engage in a positive feedback mechanism and treatment of rat hippocampal neurons with AMPA and NMDA, ligands for the glutamate receptors AMPAR and NMDAR, increases mitochondrial-derived superoxide generation [26,27].

Treatment of hippocampal slices with a superoxide-generating system induces CA1 LTP, application of a superoxide dismutase (SOD) mimetic (neutralizes ROS) prevents CA1 LTP induced by high frequency stimulation, and overexpression of SOD prevents CA1 LTP in transgenic mice [28–30]. Metformin also has also been shown to activate AMPK in hippocampal CA1 pyramidal neurons, indicating that cellular stress (mediated by ROS, Ca2+, and/or AMP(ADP)/ATP ratio increases) is critical for the induction of hippocampal CA1 LTP [31]. Interestingly, neurons of the thalamus have been shown to release glutamate in the prefrontal cortex and the primary sensory cortices (e.g. visual, auditory, and somatosensory cortices), the NMDAR facilitates LTP of thalamic neurons that synapse onto neurons located in the primary visual cortex, and NMDA activates AMPK in cortical neurons [32,36–38].

Marinangeli et al. recently showed that the AMPK inhibitor compound C severely impaired hippocampal CA1 LTP and significantly reduced the expression of the immediate early genes Arc, Egr1, and cFos after synaptic activation [188]. Compound C also blocked long-term memory formation in mice during an inhibitory avoidance task, indicating that AMPK activation is likely required for LTP, learning, and memory [24,188]. Furthermore, similar to the critical role AMPK plays in facilitating LTP, AMPK is also activated in cortical neurons by glutamate, the primary excitatory neurotransmitter in the human brain, indicating that AMPK activation may also represent a key mechanism within the neural correlates of consciousness [32]. Potassium chloride (KC), which is widely used to induce neuronal depolarization, also activates AMPK in rat cortical neurons [32]. Chen et al. demonstrated that depolarization and excitation of rat visual cortical neurons with KC led to a rapid activation of AMPK and a significant increase in ATP production as well as the mRNA expression levels of NRF-2α, PGC-1α, and mtTFA, proteins that play critical roles in regulating and enhancing mitochondrial oxidative phosphorylation and mitochondrial biogenesis [33,34]. AICAR and resveratrol also activated AMPK and increased ATP levels and the expression levels of NRF-2α whereas the AMPK inhibitor compound C blocked KC- and resveratrol-induced increases in NRF-2α, PGC-1α, mtTFA, and ATP [33]. Metformin was also shown to inhibit respiratory chain complex I activity in rat primary cortical neurons and protect neurons from etosopide-induced cell death, strongly indicating that cellular stress-induced AMPK activation resulting from mild complex I inhibition is neuroprotective in cortical neurons [35].

Moreover, similar to the activation of AMPK in cortical neurons by glutamate, nearly every neurotransmitter released from arousal circuits that promotes wakefulness, arousal, and consciousness activates AMPK, including histamine, dopamine, acetylcholine, norepinephrine, and orexin-A [39–43]. Serotonin also likely activates AMPK as Laporta et al. showed that the serotonin precursor 5-hydroxytryptophan induces AMPK activation in vivo in rats [44]. As such, and because potentiation of chloride influx through the GABAA receptor is a primary mechanism by which anesthetics including propofol and sevoflurane promote LOC, it would be expected that GABA receptor inhibitors that reverse GA would activate AMPK. Indeed, the GABA receptor antagonist bicuculline reverses propofol anesthesia and activates AMPK in mouse cortical neurons [45,46]. Stimulation of primary mouse cortical neurons with bicuculline (via neuronal disinhibition) led to an increase in synaptic activity and a rapid and sustained activation of AMPK mediated by Ca2+ influx through NMDARs and L-type voltage-gated calcium channels [46]. Flumazenil, a GABA receptor inhibitor that also accelerates emergence from sevoflurane GA in humans, protects myocytes by acting as a preconditioning agent via increasing the levels of ROS, suggesting that flumazenil may promote accelerated emergence from anesthesia primarily by activating AMPK [47,48]. Additionally, bicuculline, flumazenil, and the GABA receptor inhibitors picrotoxin, pentylentetrazol, and bilobalide ameliorate deficits in memory and hippocampal LTP in mouse models of Alzheimer’s disease and Down syndrome (DS) [49–51]. A combination of resveratrol and EGC (AMPK activators found in various plants) improved normal progenitor cell proliferation in a DS mouse model and treatment of human fibroblasts derived from DS fetuses with metformin reversed mitochondrial defects, suggesting that AMPK activation likely represents a common mechanism through which diverse GABA receptor antagonists elicit various therapeutic effects [52–54].

As discussed above, GABA and galanin release from VLPO neurons contribute to anesthetic-induced LOC via inhibition of various arousing nuclei associated with wakefulness. Expectedly, VLPO neurons are also active during non-rapid eye movement (NREM) sleep, a phase of physiological sleep that displays EEG patterns similar to sedation induced by dexmedetomidine [3,55]. As similar neural circuits are involved in both sleep and GA, changes in energy metabolism and AMPK activation should also be evident during sleep and wakefulness. Dworak et al. showed that during the initial periods of sleep in rats, ATP levels increase significantly in the frontal cortex and basal forebrain, two areas of the brain that play a critical role in anesthetic-induced LOC [56]. NREM delta activity (0.5–4.5 Hz) was also significantly higher during the initial stages of sleep. Short-term sleep deprivation however attenuated the increases in ATP levels in several brain regions including the frontal cortex and lateral hypothalamus, indicating that short-term sleep deprivation or extended periods of wakefulness represents an endogenous cellular stressor [56]. As increases in the AMP(ADP)/ATP ratio activate AMPK, short-term sleep deprivation also significantly increased AMPK activation in the basal forebrain compared to control animals [56]. Nikonova et al. further confirmed that sleep deprivation increased the mRNA levels of nuclear respiratory factors Nrf1 and Nrf2, mitochondrial electron transport chain complex IV proteins, and phosphorylated/activated AMPK in the mouse cerebral cortex [57]. Short-term sleep deprivation also increases hippocampal neurogenesis in rats and exerts antidepressant effects in humans, indicating that cellular stress-induced AMPK activation likely represents a common mechanism linking learning and memory with wakefulness, arousal, and consciousness [24,58,59].

Moreover, creatine, an amino acid that increases exercise performance, significantly decreases total sleep time and ATP levels in the cerebral cortex and basal forebrain and reduces NREM delta activity in rats [60]. Creatine has also been shown to activate AMPK in rat skeletal muscle cells [61]. Furthermore, treatment of rats with a combination of the anesthetic drugs ketamine and xylazine led to a LORR (i.e. surrogates for LOC in humans) and a significant increase in ATP levels in the frontal cortex and basal forebrain that positively correlated with an increase in EEG delta activity, mirroring results obtained during initial
stages of sleep in rats [62]. Xylazine, an analog of the anesthetic clonidine, induces sedation by acting as an agonist of the α2-adrenergic receptor, similar to dexmedetomidine [63]. Curiously, however, both ketamine and clonidine have been shown to induce AMPK activation and xylazine activates AMPK in the rat cerebral cortex, hippocampus, thalamus, and cerebellum [64–66]. As further explained below, nearly every anesthetic used clinically to induce LOC in humans activates AMPK, suggesting that AMPK may promote anesthetic-induced paradoxical excitation and possibly facilitate a restoration of consciousness.

**Anesthetics induce paradoxical excitation, activate AMPK, and act as preconditioning agents**

Although anesthetic drugs effectively induce LOC at appropriate doses, nearly every general anesthetic used clinically to induce LOC in humans may also induce paradoxical excitation at low doses [3]. As the name implies, paradoxical excitation is a phenomenon in which an anesthetic that is used to promote LOC instead induces behavioral indications of neuronal excitation on initial administration (e.g. eccentric body movements) as well as transient increases in EEG beta activity [3,6]. Intriguingly, healthy adult volunteers also exhibit prominent EEG beta activity just before emergence from general anesthesia and Friedman et al. showed that brain concentrations at the EC50 for induction (i.e. LORR) for both halothane and isoflurane are significantly greater than at emergence for both anesthetics in wild-type mice [67,68]. Similarly, estimated propofol concentrations at the EC50 for induction were significantly greater than at emergence in human neuromuscular patients [189]. Serum concentrations of propofol were also shown to be higher at loss of responsiveness compared to recovery of responsiveness in human volunteers [190]. Such evidence indicates that a decrease in the concentration of an anesthetic in the brain to a low, stimulatory level after removal of anesthesia may explain the increase in beta activity just before return of consciousness, similar to low-dose anesthetic-induced paradoxical excitation.

Anesthetic-induced paradoxical excitation has also been demonstrated in non-mammalian organisms, suggesting that a common mechanism underlying this phenomenon may cross species boundaries. Exposure of the nematode *C. elegans* to volatile anesthetics for example initially results in a paradoxical increase in movement, later followed by a progressive lack of coordination, immobility, and ultimately unresponsiveness. Removal of the anesthetic however quickly leads to a return of motion [69,70]. Loss of neural AMPK (sak-2 in *C. elegans*) inhibits movement whereas isoformurane acts as a preconditioning agent in *C. elegans* [71,72]. Additionally, the anesthetic drug diethyl ether was recently shown to induce a “sedation-like” effect in plants, epitomized by a lack of response to a stimulus that normally induces movement in the Venus flytrap [73]. Preliminary data however demonstrated that the production of ROS by cold (i.e. room-temperature) plasma induced activation and trap closing of the Venus flytrap [191]. Indeed, anesthetics and increases in ROS have also been shown to promote seed germination (analogous to paradoxical excitation) and AMPK (SnRK1 in plants), ROS, and Ca2+ promotes pollen germination and fertilization in *Arabidopsis thaliana*, indicating that cellular stress-induced AMPK activation likely facilitates neuronal activation and paradoxical excitation in humans [74–77].

As noted above, the anesthetic clonidine activates AMPK in vivo in mice and the clonidine analog xylazine activates AMPK throughout the rat brain in vivo, suggesting that AMPK activation may be a common mechanism shared by low-dose anesthetic administration [64,66]. Propofol, the most widely-used anesthetic to induce and/or maintain general anesthesia in humans, was recently shown to induce AMPK activation in both mouse and human cells. Chen et al. showed that exposure of C2C12 mouse myoblast cells to propofol led to an increase in cytoplasmic Ca2+ concentrations facilitated by IP3R-mediated Ca2+ release from the ER [78]. Propofol also significantly increased intracellular ROS production which was partially mitigated by the ER stress inhibitor TUDCA, indicating that intracellular Ca2+ increases likely potentiate increases in ROS production [26,78]. Importantly, propofol enhanced cellular viability and autophagy, induced phosphorylation and activation of AMPK, but inhibited activation of mTOR (a serine/threonine kinase that inhibits autophagy) [78]. Similar results were also observed in human cells. Treatment of HeLa human cervical cancer cells with propofol stimulated ER stress and increased intracellular Ca2+ levels and autophagosome accumulation [79]. Propofol also activated AMPK and inhibited mTOR whereas ER stress and the expression of autophagy markers were abrogated by TUDCA, indicating that propofol promotes AMPK activation in various cell types via the induction of cellular stress [79].

Additionally, clinically relevant concentrations of propofol increases differentiation of rat hippocampal-derived neuronal precursor/stem cells and propofol elevates cytoplasmic Ca2+ concentrations and promotes proliferation of adult rat neural stem cells [80,81]. Propofol in clinically relevant concentrations has also been shown to stimulate human cortical neural progenitor cell proliferation and differentiation into a neuronal cell fate via IP3R-mediated Ca2+ release from the ER [82]. Propofol also improved cellular proliferation and exerted a neuroprotective effect in human neuroblastoma SH-SY5Y cells [83]. Intriguingly, propofol has been shown to stimulate the receptors TRP1 and TRPV1, increase intracellular Ca2+ levels, and paradoxically activate nociceptive sensory neurons, contributing to the well-documented injection-site pain on initial propofol administration [84]. AITC and capsaicin, agonists for TRP1 and TRPV1, respectively, both activate AMPK, providing further evidence that low-dose anesthetic-induced AMPK activation likely plays a critical role in neuronal activation, paradoxical excitation, and consciousness [85,86].

Other structurally diverse anesthetic drugs have also been shown to induce AMPK activation. The volatile anesthetic sevoflurane demonstrated cardioprotective effects against ischemia/reperfusion (I/R) injury in isolated rat hearts via ROS-induced AMPK activation [87]. Sevoflurane preconditioning increased ROS and activated AMPK in vivo in rats and significantly reduced infarct size following myocardial I/R in an AMPK-dependent manner [88]. Sevoflurane also induced an IP3R-mediated increase in intracellular Ca2+ levels in a cholinergic cell line and enhanced aversive memory formation in rats at low doses [89,90]. Preconditioning of mice with the inhaled anesthetic isoflurane attenuated liver IR injury in an AMPK-dependent manner and low doses of isoflurane promote proliferation and differentiation of human cortical neural progenitor cells via IP3R-mediated increases in intracellular Ca2+ levels, similar to propofol and sevoflurane [91,92]. The dissociative anesthetic ketamine was shown to activate AMPK in the rat hippocampus in vivo and the rapid antidepressant effect of ketamine was attenuated by an AMPK antagonist [93]. Ketamine also activates Ca2+ channels in rats to induce antidepressant effects, increases ROS in mouse brain, enhances hippocampal LTP at CA3–CA1 synapses in adult rats, and reduces symptoms of depression and suicidality in human patients [94–97]. Dexmedetomidine inhibits cerebral I/R-induced neuroinflammation in rats via AMPK activation, midazolam activates AMPK in vivo in rats, and thiopental (a barbiturate anesthetic) ameliorates hypoxic cell damage and activates AMPK in human neuronal SK-N-SH cells [98–100]. Additionally, the local anesthetic lidocaine suppresses neuroinflammation and alleviates morphine tolerance via AMPK activation in the mouse spinal cord, indicating that AMPK activation represents a common mechanism linking the beneficial effects associated with low-dose anesthetic administration [101].

Several general anesthetics also function as preconditioning agents for a variety of cell types. Preconditioning refers to the exposure of a cell or an organism to a mild or sublethal stressor that leads to an adaptive response and protection against a subsequent and potentially lethal application of the same or a similar stressor [102,103]. Numerous compounds and methodologies exert preconditioning effects or act as preconditioning agents, including fasting, exercise, hydrogen sulfide, and hydrogen peroxide [102,103]. Because the induction of cellular
stress plays a critical role in neuronal activation and paradoxical excitation and because AMPK is present in neurons throughout the brain, it is likely that a primary mechanism through which general anesthetics function as preconditioning agents is via cellular stress-induced AMPK activation. Temperature preconditioning of rat myocytes leads to a cardioprotective effect that is dependent on increases in mitochondrial ROS production and the ROS scavenger NAC inhibits hypoxic preconditioning-induced improvement of human adipose stroma/stem cell angiogenic capacities [104,105]. Ca2+ preconditioning also protects human myocardium against I/R injury and protects rat hearts against post-ischemic myocardial dysfunction [106,107].

Expectedly, activation of AMPK also plays a central role in preconditioning-induced neuroprotection. Cortical spreading depression (CSD) is a phenomenon characterized by a wave of neuronal depolarization in the cortex followed by a period of quiescence that is associated with increases in metabolic rate and cerebral blood flow [108]. CSD may be induced experimentally by electrical stimulation or treatment with KCl, has been found to occur in the human cerebral cortex, and CSD-induced preconditioning of the brain enhances tolerance to subsequent ischemic insults [108,109]. Viggiano et al. showed that CSD induced by KCl (which activates AMPK in visual cortical neurons) led to a significant increase in AMPK activation which was predominately associated with neurons in the superficial layers of the cerebral cortex in rats [33,110]. KCl-induced CSD preconditioning also activated AMPK in the rat cortex and significantly alleviated neurological deficits in a middle cerebral artery occlusion I/R injury model that was mitigated by an autophagy inhibitor [111]. The AMPK inhibitor compound C however downregulated the protein levels of the autophagy markers ULK1 and LC3-II, indicating that the neuroprotective effects of CSD preconditioning are likely AMPK-dependent [111]. Additionally, ischemic preconditioning-induced neuroprotection was shown to be AMPK-dependent in a rat model of ischemic stroke and the AMPK activator AICAR protects primary mouse cortical neurons from NMBA-induced excitotoxicity, providing further evidence that AMPK activation plays a central role in stress-induced preconditioning of the brain [112,113].

Because nearly every neurotransmitter that promotes wakefulness, arousal, and consciousness activates AMPK and because nearly all general anesthetics used clinically activate AMPK, it would be expected that both neurotransmitters and anesthetics also act as preconditioning agents. Such a notion would also imply that cellular stress-induced AMPK activation is a common mechanism that potentially underlies and links preconditioning with paradoxical excitation, accelerated emergence from anesthesia, and restoration of consciousness (discussed in the next section). Indeed, glutamate preconditioning of rat cortical neurons exerts a neuroprotective effect against oxygen/glucose deprivation, acetylcholine-mediated preconditioning reduces infarct size in a rat model of I/R injury, and dopamine preconditioning significantly reduces infarct size in hyperlipidemic rat hearts [114–116]. Endogenous histamine mediates hypoxic preconditioning-induced stroke tolerance in mice, norepinephrine-induced preconditioning of rat hearts is cardioprotective in an I/R injury model, and serotonin protects mouse cortical neurons from methylmercury-induced cytotoxicity [117–119].

Preconditioning with the widely-used general anesthetic propofol exerts a protective effect against I/R injury in human hepatocytes, sevofoflurane preconditioning enhances endogenous neurogenesis in the subventricular zone and is neuroprotective in a rat model of ischemic brain injury, and short-term isoflurane preconditioning protects rat primary cortical neurons against neurotoxicity induced by subsequent longer exposures to isoflurane [120–122]. Strikingly, slow intravenous injections of low-dose propofol to adult surgical patients resulted in low-pain intensity whereas venous occlusion-induced sustained exposure to propofol did not prolong pain, indicating that low-dose propofol may act as a preconditioning agent to reduce injection-site pain resulting from subsequent high-dose propofol injections [135]. Low-dose ketamine preconditioning also exerts a protective effect after induction of pneumoperitoneum (presence of gas or air in the abdominal cavity) in rats, dexmedetomidine preconditioning of rat brain corticale cultures demonstrated neuroprotective effects in an in vitro model of cerebral ischemia, and midazolam preconditioning enhances the protective effect of human bone marrow-derived mesenchymal stem cells in a rat model of hepatic I/R injury [123–125]. The GABAA receptor antagonists flumazenil and bicuculline, the anesthetic gas xenon, and the opioid analgesic remifentanil (commonly used during general anesthesia) each act as preconditioning agents, indicating that xenon, remifentanil, and flumazenil are also AMPK activators [48,126–128].

Recent evidence also suggests that various general anesthetics may share a common mechanism with the anti-diabetic drug metformin to induce AMPK activation. Similar to propofol and sevoflurane, metformin increases intracellular Ca2+ and ROS levels and acts as a neuroprotective preconditioning agent against focal cerebral ischemia in an AMPK-dependent manner [129–131]. A well-studied mechanism through which metformin induces AMPK activation is through mild inhibition of complex I of the mitochondrial electron transport chain (ETC), leading to the induction of cellular stress [19]. Mutations in a subunit of complex I of the mitochondrial ETC in C. elegans leads to immobilization (analogous to LOC and LORR) at lower concentrations of volatile anesthetics compared to wild-type animals, suggesting that in addition to the GABAA and NMDA receptors, the mitochondrial ETC is a potential target of anesthetic drugs [132]. AMPK activation may result from mitochondrial ETC inhibition, as C. elegans exhibits a paradoxical increase in movement when initially exposed to volatile anesthetics whereas a loss of neural AMPK/aak-2 inhibits movement [69–71]. A similar effect has also been observed in mice, where inactivation of a subunit of complex I of the mitochondrial ETC led to a 2.5–3.0 fold increase in sensitivity to volatile anesthetics (loss of response to tail clamp) and a 2-fold increase in sensitivity to propofol (LORR) compared to wild-type mice [133]. Propofol has also been shown to inhibit mitochondrial ETC function and increase ROS levels in human neuroblastoma SH-SY5Y cells, effects that were enhanced via the addition of metformin [136]. Inhibition of mitochondrial function contributes to the pathophysiology of propofol infusion syndrome (PRIS), a potentially fatal condition associated with prolonged exposure to propofol in high doses [137,138]. Additionally, Morgan et al. showed that pediatric patients exhibiting mitochondrial deficiencies including defects associated with complex I of the ETC displayed hypersensitivity to sevoflurane, providing further evidence that the mitochondrial ETC is a likely anesthetic target and that mild inhibition of the ETC by low-dose anesthetics may induce beneficial AMPK activation, possibly contributing to both preconditioning and paradoxical excitation [134].

Compounds & anesthetics that accelerate emergence from anesthesia and potentially enhance recovery from disorders of consciousness likely do so via AMPK

Unlike induction, emergence from GA is a passive process that occurs after removal of anesthesia and can be assessed by a patient’s response to a verbal command [3,7,8]. Because nearly every neurotransmitter that promotes wakefulness, arousal, and consciousness activates AMPK (i.e. glutamate, acetylcholine, histamine, orexin-A, dopamine, and norepinephrine), it is likely that cellular stress-induced AMPK activation in neurons plays a pivotal role in accelerating emergence from anesthesia and restoring consciousness [32,39–43]. Evidence that stress mediates and enhances the activity of neurotransmitters is abundant in the plant kingdom. Although they do not have a nervous system, plants produce nearly all neurotransmitters (i.e. glutamate, acetylcholine, histamine, dopamine, serotonin and norepinephrine) that are critical for maintaining consciousness in humans and biotic (e.g. predator attack) and abiotic (e.g. drought) stressors have been well-described to increase the production and activity of these neurotransmitters in plants [139–141]. Toyota et al. recently demonstrated that wounding via scissors or caterpillar feeding on an
Arabidopsis plant led to an increase in both glutamate and Ca2+ with glutamate-induced activation of glutamate receptor-like ion channels generating intracellular Ca2+ concentrations that propagated to distal plant organs to induce a systemic defense response [141]. Several abiotic stressors (e.g., nutrient deficiency, osmotic and oxidative stress, etc.) also activate autophagy in Arabidopsis in an AMPK/SnRK1-dependent manner [142]. Fungal infection of certain rice cultivars increases the production of serotonin, which suppresses leaf damage and reduces biotic stress and the production of dopamine by the green alga Ulvaria obscura acts as a feeding deterrent against sea urchins, snails, and isopods [143,144]. Interestingly, just as various volatile anesthetics promote seed germination, certain plants have been shown to produce the anesthetic divinyl ether when stressed [74,145,146]. ROS and Ca2+ also play critical roles in the production of secondary metabolites, compounds that plants produce in response to stress partly for the purpose of self defense [147,148]. Moreover, the GABAA receptor antagonist bicuculline, a secondary metabolite produced by the plant Corydalis chaerophylla, reverses propofol anesthesia and activates AMPK in cortical neurons, suggesting that a mechanism of cellular stress-induced AMPK activation by neurotransmitters may have been evolutionarily conserved to promote neuronal activation in the human brain [45,46,149,150].

Several plant-derived secondary metabolites have also been shown to accelerate emergence from anesthesia in animal models and in humans. The secondary metabolites forskolin (derived from the plant Plectranthus barbatus) and caffeine (derived from numerous plants) both activate AMPK and dramatically accelerate emergence from isoflurane anesthesia in rats while caffeine also accelerates emergence from anesthesia in propofol-anesthetized rats [20,21,151–153]. Similar to the anesthetic ketamine and increases in intracellular ROS and Ca2+, forskolin also promotes LTP in hippocampal slices [25,26–30,96,154]. A recent randomized, double-blind, crossover study further demonstrated that an intravenous infusion of caffeine led to a 42% reduction in the mean time to emergence in healthy males anesthetized with isoflurane compared to placebo, indicating that compounds that activate AMPK are capable of accelerating emergence from anesthesia in humans [155]. Additionally, intrathalamic microinjection of nicotine, a well-studied secondary metabolite derived from the plant Nicotiana attenuata, reverses sevoflurane-induced unconsciousness (i.e. LORR) in rats [156,157]. Nicotine also enhances hippocampal CA1 LTP in mice and both nicotine and metformin activate AMPK in the hippocampus in vivo in mice [22,158]. Interestingly, metformin was originally derived from a secondary metabolite found in the plant Galagia officinalis [19,159]. Physostigmine, a secondary metabolite found in the plant Physostigma venenosum, increases acetylcholine levels through reversible inhibition of acetylcholinesterase and restores consciousness in human volunteers anesthetized with propofol [160,161]. Additionally, acetylcholine activates AMPK and stimulation of the prefrontal cortex with the cholinergetic agonist carbachol reversed sevoflurane anesthesia and restored wake-like behavior in rats [39,162]. As carbachol activates AMPK in human neuroblastoma SH-SY5Y cells, cellular stress-induced AMPK activation likely also plays a role in physostigmine-induced restoration of consciousness in humans [163]. Furthermore, a ketogenic diet (i.e. high fat/low carbohydrate diet) or a standard diet supplemented with the exogenous ketone body-hydroxybutyrate (βHB) significantly delayed the onset of isoflurane-induced anesthesia in rats [164]. βHB activates AMPK and a ketogenic diet transiently increases the levels of ROS in the hippocampus in vivo in rats, providing compelling evidence that cellular stress-induced AMPK activation promotes delayed induction of and accelerated emergence from anesthesia [165,166].

As glutamate and nearly every neurotransmitter released from ascending arousal circuits that promote wakefulness, arousal, and consciousness activates AMPK, it would be expected that compounds or methodologies that enhance neurotransmitter activity would also accelerate emergence from anesthesia [32,39–43]. Dextroamphetamine, which induces the release of dopamine, significantly accelerates emergence from both sevoflurane and propofol anesthesia whereas the D1 dopamine receptor antagonist SCH-23390 inhibited chloro-APB (a D1 receptor agonist)-mediated restoration of righting and emergence from isoflurane anesthesia in rats [167,168]. The selective D1 receptor agonist fenoldopam however activates AMPK in human monocytes [43]. Microinjection of orexin-A into the basal forebrain of rats also facilitated emergence from propofol anesthesia [169]. Rao et al. demonstrated that prolonged wakefulness in mice, which activates AMPK, produces LTP of glutamatergic synapses on orexin neurons. The D1 dopamine receptor antagonist SCH-23390 however attenuated synaptic plasticity whereas forskolin induced LTP in orexin neurons, providing further evidence that cellular stress-induced AMPK activation links LTP and restoration of consciousness [21,24–30,56,170].

Activation of glutamatergic neurons in the parabrachial nucleus (located in the brain stem) accelerated emergence from sevoflurane anesthesia in mice and also prolonged induction time, similar to ketone body/ketogenic diet-induced delayed onset of isoflurane anesthesia in rats [164,171]. Strikingly, propofol was recently shown to induce an abrupt increase in Ca2+ levels in parabrachial nucleus neurons in vivo just before LORR and recovery of righting reflex (RORR) in rats, indicating that propofol is excitory at low doses and promotes paradoxical excitation and possible facilitation of return of consciousness via cellular stress induction (i.e. Ca2+ and/or ROS) and AMPK activation [192]. Microinjections of histamine into the nucleus basalis magnocellularis located in the basal forebrain also facilitates emergence from isoflurane anesthesia and induces EEG arousal in rats [172]. Delivery of noradrenaline (also known as norepinephrine) into the prefrontal cortex led to EEG activation but did not induce behavioral signs of arousal in rats anesthetized with sevoflurane [162]. However, infusion of orexin-A into the locus cereuleus promotes norepinephrine-induced LTP in the dentate gyrus of the rat hippocampus and stimulation of serotonergic axons in the hippocampal CA1 region enhances spatial memory in mice, similar to nicotine, forskolin, and ketamine [96,154,158,173,174]. Moreover, as the anesthetic ketamine induces antidepressant effects in rats by activating AMPK and nearly every general anesthetic used clinically induces AMPK activation similar to neurotransmitters, it would also be expected that certain anesthetic drugs may paradoxically accelerate emergence from anesthesia [93]. Hambrecht-Wiedbusch et al. showed that injection of a single dose of subanesthetic ketamine initially increased anesthetic depth in isoflurane-anesthetized rats but also increased cortical acetylcholine release and paradoxically accelerated emergence and recovery of consciousness, again suggesting that AMPK activation represents a common mechanism linking neurotransmitter- and low-dose anesthetic-induced restoration of consciousness [175].

Lastly, cellular stress-induced AMPK activation may also prove effective for individuals affected by disorders of consciousness. Disorders of consciousness include a minimally conscious state, vegetative state, and coma and often stem from traumatic or non-traumatic injuries to the brain [176]. Patients in a minimally conscious state exhibit significantly impaired consciousness with intermittent periods of self-awareness whereas a vegetative state is characterized by wakefulness without cognition and self-awareness [3,176]. Coma is a deep state of unconsciousness characterized by abnormal brain stem reflexes and a lack of wakefulness and awareness [176]. Neurogenesis, a process of generating neurons from neural stem or progenitor cells (NSCs), has been shown to occur in the adult mammalian brain in the subgranular zone (SGZ) of the dentate gyrus in the hippocampus and in the subventricular zone (SVZ) of the lateral ventricles [177]. Cellular stress (e.g. ROS) induces differentiation of embryonic and adult stem cells, ischemia stimulates neurogenesis in the SGZ and SVZ in rats in vivo, and the SVZ lies in close proximity to the cerebral cortex [52,177,178]. Because metformin promotes neurogenesis in both the SGZ and the SVZ in vitro and in vivo, compounds that activate AMPK via the induction of cellular stress may enhance brain repair and potentially facilitate
restoration of consciousness in patients with disorders of consciousness [179–182]. Moreover, evidence also indicates that cellular stress and AMPK activation may link human consciousness with seemingly disparate physiological and pathophysiological phenomena, including accelerated aging, HIV-1, human reproduction, cancer, gene regulation (e.g. transposable elements), plasma medicine, cell cycle regulation, meditation, fetal hemoglobin induction, parabiosis (i.e. young blood), planarian regeneration, and stress-induced CRISPR-Cas activation in bacteria (e.g. gene editing technology) [24,52,183–187,193–202].

**Hypothesis testing**

AMPK knockdown or pharmacological inhibition in various brain structures (e.g. basal forebrain, cerebral cortex, thalamus, hypothalamus, brain stem, etc.) would be necessary to determine if AMPK activation is essential in facilitating accelerated emergence from anesthesia induced by certain compounds (e.g. caffeine, forskolin, methylphenidate, etc.). Appropriately designed and approved case studies using AMPK activators that possess exemplary safety profiles (e.g. metformin, caffeine, etc.) would also indicate if AMPK activation plays a beneficial role in restoration of consciousness in patients diagnosed with disorders of consciousness (i.e. minimally conscious state, vegetative state, coma).

**Conclusion**

In conclusion, cellular stress-induced AMPK activation, mediated by increases in intracellular ROS, Ca2+, and/or an AMP(ADP)/ATP ratio increase, etc. links wakefulness, arousal, and consciousness with paradoxical excitation and accelerated emergence from anesthesia (Fig. 1). AMPK is located throughout the brain and is activated by nearly every neurotransmitter that promotes arousal and consciousness, including glutamate, acetylcholine, histamine, orexin-A, dopamine, norepinephrine, and noradrenaline. Nearly every anesthetic used clinically to promote LOC in human patients induces paradoxical excitation in low doses and also activates AMPK (i.e. propofol, sevoflurane, isoflurane, dexmedetomidine, ketamine, and midazolam). Because anesthetic brain concentrations are significantly higher during induction than at emergence, cellular stress-induced AMPK activation by low doses of anesthetics and neurotransmitters link paradoxical excitation with accelerated emergence from anesthesia and return of consciousness. Additionally, several general anesthetics and neurotransmitters act as preconditioning agents and increases in ROS, Ca2+, and AMPK activation have been shown to exert preconditioning effects, indicating that paradoxical

excitation is analogous to preconditioning and both likely share a common mechanism of AMPK activation. Also, metformin, an AMPK activator with an exceptional safety profile that crosses the blood-brain barrier in vivo, induces proliferation and differentiation of neural stem cells in the dentate gyrus and subventricular zone, indicating that AMPK activation may promote brain repair and restoration of consciousness in patients diagnosed with disorders of consciousness (i.e. minimally conscious state, vegetative state, coma).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2019.01.014.

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