Minireview

A primordial target: Mitochondria mediate both primary and collateral anesthetic effects of volatile anesthetics

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Impact Statement

Understanding the mechanisms of action of volatile anesthetics (VAs) will not only reveal information about their roles as agents used worldwide to induce unconsciousness and analgesia but also the causes of the desirable and undesirable collateral effects of these agents. In addition to satisfying intellectual curiosity about the nature of consciousness, an understanding of the metabolic pathways mediating the complex effects of VAs could reveal molecular targets for therapeutic exploitation as neuroprotectants. Genetic studies in invertebrates have provided a paradigm shift by suggesting that mitochondria may harbor the molecular switch activating both primary and collateral effects. The disruption of a specific step of electron transfer within the mitochondrion causes hypersensitivity to VAs, from nematodes and fruit flies to humans while also modulating the sensitivity to collateral effects. The striking evolutionary consistency raises the possibility that an ancient mechanism is at hand, linking metabolism in prokaryotes to neuronal silencing in mammals.

Abstract

One of the unsolved mysteries of medicine is how do volatile anesthetics (VAs) cause a patient to reversibly lose consciousness. In addition, identifying mechanisms for the collateral effects of VAs, including anesthetic-induced neurotoxicity (AiN) and anesthetic preconditioning (AP), has proven challenging. Multiple classes of molecules (lipids, proteins, and water) have been considered as potential VA targets, but recently proteins have received the most attention. Studies targeting neuronal receptors or ion channels had limited success in identifying the critical targets of VAs mediating either the phenotype of "anesthesia" or their collateral effects. Recent studies in both nematodes and fruit flies may provide a paradigm shift by suggesting that mitochondria may harbor the upstream molecular switch activating both primary and collateral effects. The disruption of a specific step of electron transfer within the mitochondrion causes hypersensitivity to VAs, from nematodes to *Drosophila* and to humans, while also modulating the sensitivity to collateral effects. The downstream effects from mitochondrial inhibition are potentially legion, but inhibition of presynaptic neurotransmitter cycling appears to be specifically sensitive to the mitochondrial effects. These findings are perhaps of even broader interest since two recent reports indicate that mitochondrial damage may well underlie neurotoxic and neuroprotective effects of VAs in the central nervous system (CNS). It is, therefore, important to understand how anesthetics interact with mitochondria to affect CNS function, not just for the desired facets of general anesthesia but also for significant collateral effects, both harmful and beneficial. A tantalizing possibility exists that both the primary (anesthesia) and secondary (AiN, AP) mechanisms may at least partially overlap in the mitochondrial electron transport chain (ETC).

Keywords: Anesthetics, genetics, sensitivity, neurotoxicity, pharmacogenetics, mitochondria

***Experimental Biology and Medicine* 2023; 248: 545–552. DOI: 10.1177/15353702231165025**

Introduction

The first proposal linking anesthesia directly to the effects of respiratory enzymes was formulated in 1914 by Otto Warburg[1] within the larger framework of hypotheses of asphyxia as a mechanism of volatile anesthetic (VA) action. The asphyxic framework of anesthesia lost favor in subsequent years but the "Warburg apparatus" continued to be used in the studies of tissue respiration. In the 1960s, as the chemiosmotic hypothesis of the general mechanism of oxidative and photosynthetic phosphorylation was formulated by Peter D. Mitchell, halothane was noted to suppress tissue oxygen consumption.[2,3]

In subsequent years, better understanding of oxidative phosphorylation and refinements in experimental techniques allowed the conclusion that VAs had a direct effect on mitochondrial respiration.[4–6] Furthermore, the data suggested a specific interaction between halothane and complex

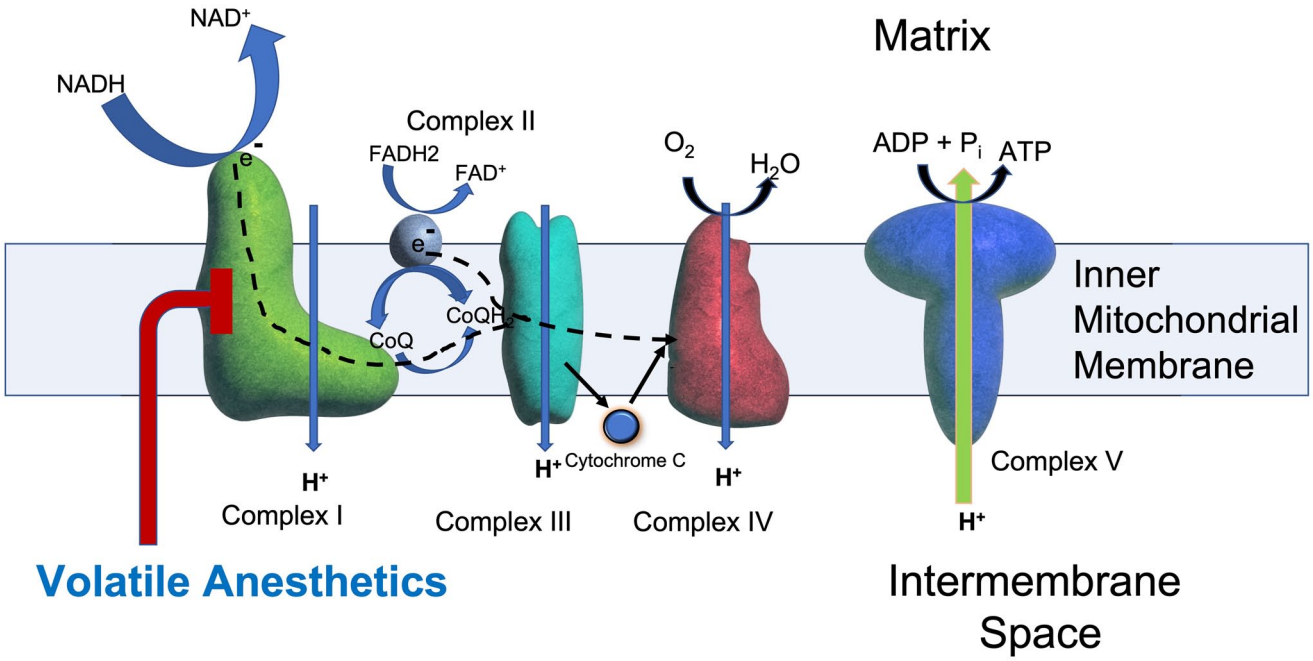

Figure 1. Complex I in the mitochondrial electron transport chain (mETC). Electrons (e^-) are donated to complex I (green) by NADH and to complex II (gray) by $FADH_2$. Both complexes I and II then donate electrons (dashed line) to co-enzyme Q (CoQ) which transfers them complex III (turquoise) and then, via cytochrome C, to complex IV (red). Complexes I, III, and IV of the mETC pump protons to serve as a proton-motive force to drive production of ATP by complex V (blue). Embedded in the inner mitochondrial membrane, complex I is a large (~46 subunits) structure that transfers electrons from NADH to ubiquinone (CoQ). In the mETC, complex I is uniquely inhibited by VAs (red line) and contains NDUFS2, NDUFS4, NDUFS8, and ND2, all proteins discussed in this review.

I (Figure 1) of the mitochondrial electron transport chain (ETC).[5] Entry of electrons via complex II of the ETC was relatively unaffected by halothane compared to the flow of electrons via complex I. More recent data with improved techniques have confirmed that complex I-dependent respiration is indeed sensitive to VAs in the "clinical range," whereas the other complexes of the ETC are not sensitive despite exposure to much higher VA concentrations.[7,8]

The overall conclusion about anesthetics and mitochondrial function was that a variety of anesthetics suppressed tissue oxygen consumption and that this effect was observed with complex I-specific substrates but not with a complex II substrate.[9,10] However, correlative studies of anesthetized tissues showed that "energy reserves" (ATP, glycogen phosphocreatine etc.) were maintained or increased[11] compared to the unanesthetized state. Further work from Michenfelder also found that ATP levels in the cortex were not decreased at high VA concentrations and after prolonged exposure.[12] Therefore, the degree to which interference with mitochondrial function was a significant contributor to anesthesia remained, at best, controversial. However, recent genetic studies have rekindled the interest in mitochondrial inhibition as a primary effect of VAs.

Genetic studies

Mitochondrial complex I and anesthetic sensitivity – invertebrate genetic models. Early work in the roundworm (*Caenorhabditis elegans*) identified several mutations which increased VA sensitivity. A mutation in one gene, *gas-1(fc21)*, increased sensitivity to all VAs but the animals moved normally in air.[13] *gas-1(fc21)* reduced the EC_{50}s of multiple VAs (isoflurane by over 80%) and shifted the dose-response curves for all VAs far to the left of wildtype. *gas-1* overrides the effects of other genes that affect VA sensitivity in *C. elegans* and as such was postulated to be the closest gene to a putative anesthetic target.[13,14]

gas-1 was cloned and found to encode the highly conserved 49-kDa subunit (aka NDUFS2) of complex I, an entry point into the mitochondrial ETC (Figure 1).[15,16] In contrast, animals with defects in complexes II, III, and IV of the ETC shared many phenotypes with *gas-1*, but, remarkably, were NOT hypersensitive to VAs.[17–20] Complex I (NADH-ubiquinone oxidoreductase) catalyzes the reduction of ubiquinone (CoQ) by transferring electrons from NADH to complex III.[16] Another mutation, *clk-1*, is defective in CoQ synthesis and increases sensitivity to VAs.[21,22] NDUFS2 is part of the CoQ binding site for complex I[23–26] pinpointing this interaction as a potential determinant of anesthetic sensitivity. An RNAi-based screen indicated that subunits residing within subcomplex Iλ, close to the CoQ binding site, most directly influenced whole-worm anesthetic behavior.[27]

The work in worms prompted investigations of mitochondrial defect-linked hypersensitivity to VAs in fruit flies (*Drosophila melanogaster*). Fruit flies have a complex CNS and roughly 70% of disease-causing genes are conserved between humans and fruit flies, the percentage being even higher in the nervous system.[28–30] In analogy to the findings in rodents and humans (see below), a model of Leigh syndrome (LS) in fruit flies was used to investigate anesthetic action. LS is a fatal neurodegenerative disease caused primarily (but not exclusively) by mutations in complex I of the ETC. The $ND23^{60114}$ line used in these experiments was generated by ethyl methanesulfonate mutagenesis in previous screens for

temperature-sensitive paralytic mutants.[31] Characterization of this line revealed that flies homozygous for the *ND23*^{60114} allele reproduce many features of LS: morphological signs of neurodegeneration as young adults, shortened lifespan, abnormal mitochondrial morphology, and reduced adenosine triphosphate (ATP) levels. The causal mutation is an SNP in the *ND23* gene (mammalian *Ndufs8*), a highly conserved subunit of mitochondrial complex I.[32] Anesthetic sensitivity was determined for isoflurane and sevoflurane using a modified rapid iterative negative geotaxis (RING) assay[33] and compared to six strains of wildtype flies (including the Canton-S, the background strain for *ND23*^{60114}).[34] The EC_{50}s for suppression of locomotion by isoflurane and sevoflurane were reduced from 0.36% and 0.89%, respectively, in Canton-S to 0.20% and 0.47%, respectively in *ND23*^{60114} flies,[34] corroborating the findings of increased VA sensitivity in patients and other animal models with complex I defects. Notably, after determining the EC_{50}s of wildtype and mutant flies at one to eight days of age, all flies recovered from the exposure without mortality. When exposed at 10 days of age, *ND23*^{60114} flies present a mortality phenotype discussed in Part III.

Mitochondrial complex I and anesthetic sensitivity – humans. In general, patients with mitochondrial dysfunction (documented by biochemical studies or by histology) tolerate general anesthesia well.[35,36] However, there were reports in the literature of poor outcomes in patients after anesthetic exposure.[37–39] These reports led to the question of whether there might be variations in anesthetic sensitivity in humans with mitochondrial defects. We questioned whether sensitivity to VAs might vary significantly in mitochondrial patients. Since sevoflurane was, and remains, the predominant VA in use in children, the concentration of sevoflurane necessary to induce loss of consciousness was correlated with mitochondrial function. Children presented with a common constellation of symptoms included hypotonia, developmental delay, failure to thrive, and lactic acidosis. The results in a small cohort of children indicated that some patients with complex I defects were extremely sensitive to sevoflurane.[40]

These results led to a larger multicenter case series of 91 patients presenting for diagnosis of mitochondrial defects.[41] Anesthesia was again induced with sevoflurane only, titrating the dose to that which caused loss of consciousness prior to surgical incision. The concentrations used to achieve this endpoint were then compared to measured function of complexes I–IV in skeletal muscle biopsies. Underlying severity of disease did not predict who might be hypersensitive to sevoflurane. The results were remarkable in that all patients with complex I dysfunction showed an increase in sensitivity to sevoflurane. However, not all patients who were hypersensitive to sevoflurane had complex I dysfunction. The authors speculated that ETC supercomplexes, consisting of complexes I–III–IV, are thought to be the functioning mitochondrial respiratory complex, and that allosteric interactions may be important for its function. However, most astonishingly, as in both the nematode and *Drosophila*, changes in complex I function uniformly resulted in hypersensitivity to sevoflurane.

Mitochondrial complex I and anesthetic sensitivity – mice. Recently, the importance of mitochondria for VA sensitivity was extended to a rodent model, the mouse, in which further physiologic studies were possible. *Ndufs4* encodes one of over 40 subunits of mammalian mitochondrial complex I.[15,42,43] The knockout (KO) of the nuclear gene *Ndufs4* exhibited large increases in behavioral sensitivity (resistance to tail clamp, loss of righting reflex) to halothane and isoflurane.[44,45] For both endpoints, the EC_{50}s of the KO for isoflurane and halothane were 1/3–1/2 those of the controls.[44] Clearly, the effects of complex I dysfunction on VA sensitivity are also profound in mice as in the nematode, fly, and human. Importantly, and frankly surprisingly, *this mitochondrial defect causes a resistance to ketamine*, indicating that the behavioral change is not simply the result of baseline nonspecific decreases in CNS function. Since complex I dysfunction causes VA hypersensitivity in organisms from nematodes to man, the implication is that an ancient mechanism is at hand, linking movement of electrons within the ETC (Figure 1) to neuronal silencing in the presence of VAs. The data indicate that complex I function is central to VA sensitivity and is likely an important VA target.

There are many possible links between mitochondrial function and neurotransmission.[46] For example, mitochondria are the site of both glutamate synthesis and the degradation of GABA, which is synthesized by decarboxylation of glutamate.[47] *In addition, complex I represents the rate-limiting step for energy production in the mitochondrion*.[48,49] Synaptic transmission is energetically demanding; excitatory neuronal activity has been estimated to account for 75% of the energy requirement for cerebellum and 50% of the cortex.[50,51] Inhibition of excitatory neurotransmission (cholinergic or glutamatergic) has been postulated as a mechanism by which VAs work and could be most sensitive to defective mitochondrial function.[46,52,53] This is especially true of glutamatergic neurotransmission, which has been shown to be the most energetically demanding neurotransmission in the CNS.[50,54,55] However, in addition to energy production, the roles of mitochondria/complex I in other important phenomena, such as ROS signaling and calcium homeostasis, make it easy to implicate them in the most important facets of neuronal signaling, both desired and collateral.

Several laboratories have shown that presynaptic function of excitatory neurons is inhibited by VAs.[56–61] Studies with wildtype and cell-specific *Ndufs4(KO)* mice showed that mitochondrial function in glutamatergic neurons determined VA sensitivity to tail clamp and loss of righting reflex.[61,62] Furthermore, presynaptic frequencies of excitatory signaling were inhibited by VAs at concentrations approximating the whole animal EC_{50}s of wildtype and *Ndufs4(KO)* mice.[61,62] The sensitivity of presynaptic frequencies to isoflurane increased when neurons were made dependent on mitochondrial respiration.

Synaptic neurotransmitter recycling, in particular endocytosis, is uniquely dependent on ATP availability.[63,64] Since VAs are known potent inhibitors of mitochondrial complex I,[5,6,8,11] its inhibition may underlie the presynaptic changes caused by VAs. Recent work in neuronal cultures demonstrated that isoflurane specifically inhibited neurotransmitter

endocytosis and ATP production at a concentration approximating the whole animal EC_{95} of wildtype mice. In addition, a similar inhibition was seen in *Ndufs4(KO)* at lower concentrations of isoflurane matching the EC_{95} of the mutant.[65] When ATP levels were supported by increasing glucose (to increase glycolysis) or use of a complex II-specific substrate to bypass inhibition of complex I, endocytosis and ATP levels were not affected by isoflurane exposure in either wildtype or mutant cultures. The results indicated that isoflurane inhibition of complex I leads to ATP depletion at the presynapse resulting in a failure of neurotransmitter cycling.[65]

To establish that complex I is a primary component controlling VA sensitivity, a change in complex I leading to resistance would be invaluable. The recent availability of a genetic model that can bypass complex I (*NDi1*)[66,67] as an electron donor allowed for direct analysis of the mitochondrial effects of isoflurane on both whole animal behavior and endocytosis. The presence of *NDi1* in the CNS of mice led to a ~25% resistance to isoflurane and halothane in loss of righting reflex (LORR) and tail clamp assays, while rescuing both the ATP decrease and failure of endocytosis in the presence of isoflurane.[65] The behavioral effects of NDi1 on VA resistance were reproducible in flies using a locomotion assay (RING) (MP, unpublished data).

VAs and metabolism

In addition to the effects of VAs on the mitochondrial ETC,[5,6,8,11] other effects of these drugs on metabolism have been also noted. Work from the Klein laboratory noted increased lactate levels in CNS tissue from animals anesthetized with VAs but not those anesthetized with parenteral agents.[68,69] Follow-up work from this group recently showed that the increase in lactate was in turn related to complex I inhibition by the VAs, specifically by halothane and isoflurane and, as they note, to a lesser extent by sevoflurane.[7]

Preliminary work from the Johnson laboratory has shown that neonates may carry an additional metabolic risk from VA exposure.[70,71] Unexpectedly, they found that in neonates blood, β-hydroxybutyrate was rapidly depleted by VAs at concentrations well below those necessary for anesthesia, while adult levels were unaffected. Depletion of β-HB was accompanied by an increase in lactate, citrate accumulation, malonyl-CoA production by acetyl-CoA carboxylase, and inhibition of fatty acid oxidation. Acylcarnitines were also depleted but the decrease was not dependent on complex I inhibition but was most consistent with an effect on carnitine-palmitoyltransferase-1 resulting in a defect in transport of fatty acids across the mitochondrial membrane. Broad changes in metabolism have also been hinted at in other studies of the isoflurane-induced changes in a metabolome.[72] While it is not yet possible to specifically identify the pathways most affected by VAs, they have broad effects; the specific pathways affected may change during development.

An extensively studied VA, isoflurane, has long been known to be effective in preconditioning tissue from exposure to hypoxia. The mechanism, not fully understood, is felt to involve metabolic inhibition (probably of mitochondrial complexes I and III)[73,74] with resulting release of reactive oxygen species (ROS).[73,75] Recent work from the AN laboratory indicated that this may involve an indirect effect of isoflurane on complex II.[76] If so, this widens the range of important effects of VAs on the ETC.

Genetic determinants of collateral anesthetic pharmacodynamics

Exposure to VAs carries the risk of anesthetic-induced neurotoxicity (AiN). Situations in which a "fragile" (developing, diseased, injured) brain is exposed to VAs increase the AiN risk.[77] Understanding of risk factors and mechanisms of toxicity is still rudimentary, and progress is slowed by cost, animal welfare concerns, and limited throughput due in part to frequently ambiguous behavioral phenotypes of mammalian models.[77,78] An important question is whether AiN is purely a xenotoxic phenomenon (anyone can be affected by an exposure to an appropriate dose of the toxin) or whether biological factors other than age determine the individual risk (i.e. could individuals at high risk be identified and protected?). Phrased differently, do genetic and environmental backgrounds determine the risk of an individual to be negatively affected by AiN? Genetic determination of risk from exposure to VAs is not unknown: due to its fulminant phenotype and Mendelian inheritance pattern, Malignant Hyperthermia Syndrome remains the best known pharmacogenetic disorder in perioperative medicine.[79] The core anesthetic phenotype of VA sensitivity, using inhibition of movement in response to a noxious stimulus, is controlled by genetic background.[80–84] It is therefore plausible that AiN is also a quantitative genetic trait. Mitochondria are extremely genetically complex organelles with a unique inheritance pattern (contributions from the nuclear genome collaborate with purely maternally inherited genes of the mitochondrial genome). High rates of mutations and mitochondrial damage have been documented in the context of AiN[85,86] and numerous neurodegenerative diseases.[87]

Due to its experimental flexibility, advanced and well-characterized neurocircuitry, and the availability of an unmatched genetic toolbox, the fruit fly is widely used as (1) a model for neurodegenerative diseases; (2) for toxicologic studies; and (3) for genetic studies and can be arguably considered the ideal model to test the hypothesis that AiN is a quantitative trait. Two models were used to explore the pharmacogenomics of the interactions of isoflurane with "fragile" brains: (1) the brain affected by neurodegeneration using a fly model of LS and (2) the injured brain using a fly Traumatic Brain Injury (TBI) model.

Mitochondrial complex I mutations predispose to AiN

Exposure of neonate and young "wildtype" (i.e. not harboring known mutations) rodents to general anesthetics causes morphological changes in mitochondria.[88] In a seven-day-old rat pups, a 6-h exposure to commonly used sedatives and anesthetics (midazolam, nitrous oxide, isoflurane) resulted in a disturbed balance between mitochondrial fission and fusion with excessive fission.[86] This impaired mitochondrial homeostasis, attributed to excessive production of ROS, was linked to the neurotoxic consequences of anesthesia.[89,90] The injectable anesthetic propofol, while less extensively

studied than isoflurane, also has toxic mitochondrial effects.[91,92] Long-term infusions of propofol can cause the propofol infusion syndrome possibly unmasking latent mitochondrial disease.[93] Short administrations have also been associated with alterations of mitochondrial dynamics *in vitro*.[85]

To examine the interaction of VAs with mitochondria, we used a fly model of LS caused by a hypomorphic mutation in the *ND23* subunit (*Ndufs8* in mice) of mitochondrial complex I. In addition to behavioral hypersensitivity, carriers of the $ND23^{60114}$ allele present an age- and O_2-dependent anesthetic toxicity phenotype. Wildtype fruit flies recover from 4%h isoflurane (concentration anesthetic × duration of exposure),[34] with no mortality and 10–20%h isoflurane causes less than 3% mortality in the wildtype. In contrast, exposure of $ND23^{60114}$ flies to 4%h isoflurane in room air results in ~50% mortality within 24 h after emergence from anesthesia.[34] Notable features of the mortality phenotype in $ND23^{60114}$ flies are: (1) age-dependence (no mortality until 10 days of adult life); (2) O_2 concentration-dependence: 75% O_2 and 5% O_2 during isoflurane exposure increases and suppresses 24 h mortality, respectively; (3) mortality can be rescued by overexpression of wildtype *ND23* in neurons; (4) heterozygous carriers of $ND23^{60114}$ develop a mortality phenotype after 4%h of isoflurane at an advanced age and only under hyperoxic conditions. Preliminary data indicate that mutations in certain other subunits of complex I also sensitize to AiN to different degrees (Borchert *submitted*). We conclude that flies used as models of LS offer an easily scored and unambiguous phenotype that in combination with the available genetic tools can be used to effectively investigate collateral effects of VAs.

Genetic background influences the extent of AiN in a fly TBI model

The availability of inbred collections of fully sequenced fly lines (e.g. the Drosophila Genetic Reference Panel [DGRP])[94] presents an opportunity to directly test the hypothesis that VA pharmacodynamics for AiN are shaped by naturally occurring genetic polymorphisms. Segregating variation in the DGRP collection mimics genetic variation in human populations, allowing inferences on the involvement of conserved pathways.[94–96] Schiffman *et al.*,[97] subjected young adult flies from 146 DGRP strains to a standard TBI protocol, followed by exposure to 1 h of 2% isoflurane in 75% O_2. Compared to paired strains in room air, treatment resulted in variable degrees of increased 24 h mortality across the lines (see supplementary figure in reference for an illustrative example). Genome-wide, five SNPs in three biologically plausible genes (*Prip*, *Drip*, and *Gyc88E*) were associated with variability in mortality at a *p*-value threshold $< 10^7$. *Prip* and *Drip* are orthologous to mammalian water permeable channels (aquaporins) and *Gyc88E* is orthologous to the oxygen sensor *GUCYB1*. Variants in the aquaporin-4 channel are associated with alterations in outcomes from various types of brain injury in rodents[98–100] and humans.[101,102] The identification of these genes using genome-wide association study (GWAS) analysis of a *Drosophila* collection attests to evolutionary conservation of key physiological pathways and to the potential for translatability of experimentally

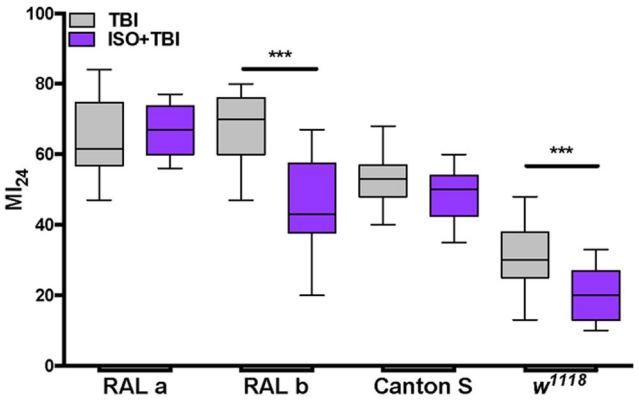

Figure 2. Genetic background influences the effectiveness of AP. Comparable mortality at 24 h after TBI (MI_{24}) in two inbred fly lines (RAL a, RAL b) and different mortality in two standard laboratory fly lines (Canton-S and w^{1118}) are shown in gray. Preconditioning (15 min with 2% isoflurane prior to TBI) suppressed the MI_{24} in RAL b and in w^{1118} but not in RAL a and Canton-S (purple). For each line and condition $N = 10–20$ vials with 20–30 flies each. Box 25th–75th percentiles, line indicates median.
$^{***}p < 0.0005$.

flexible model organisms. *GUCYB1* has not yet been associated with outcome variability in mammalian TBI models. However, the experimental paradigm used includes hyperoxia and therefore makes a cellular oxygen sensor a plausible potential candidate gene for modulating outcome of TBI. In summary, the analysis of post-TBI toxicity using tools available in the fruit fly provides proof of principle that AiN is a quantitative trait and that identification of genes contributing to AiN can be accelerated by including fruit flies into research strategies.

As a counterpoint to AiN, VAs are also known to robustly protect the brain and other tissues from damage when administered prior to ischemic injury, a phenomenon referred to as anesthetic preconditioning (AP). A detailed understanding of AP mechanisms would yield valuable targets for cerebral protection. However, because anesthetics directly modulate injury, their use in experimental brain injury deprives the experimenter of a valid (i.e. unanesthetized) control and no feasible protective strategies against TBI have been proposed to date. Genetic variability, however, is a largely unexplored variable present in the human population but seldom considered in model animal studies because of cost.[103–105] Similarly, throughput is low because of the time and effort needed to study each animal across its lifespan, which is desirable as pharmacodynamics, may be shaped by age-related changes that affect mitochondria.[97]

AP can be reproduced in a *Drosophila* closed-head TBI model caused by blunt trauma. Using mortality at 24 h after TBI (MI_{24}) as read-out, AP with the VAs isoflurane and sevoflurane substantially reduced the MI_{24}.[34] AP in the fly TBI model replicated findings obtained in mammals in that in old flies and in flies rendered obese by starvation selection over generations,[106] the effectiveness of preconditioning was thwarted.[97,107] Ongoing research suggests that, analogously to AiN, the protective effect conferred by AP in TBI varies in magnitude among DGRP lines, indicating that susceptibility to prophylactic pharmacologic brain protection is a quantitative trait. (Figure 2, unpublished observation). Once these

experiments are completed, a GWAS analysis will identify genes and pathways associated with effective AP informing strategies aimed at identifying drug targets for prophylactic pharmacological brain protection.

Summary

The important targets for VAs have remained a point of debate for over a century. While neuronal receptors and gated ion channels have recently been favored proposed targets, proof of their behavioral importance has been elusive. Mitochondria are also known to contain molecular targets of VAs and have long been known to be inhibited by these drugs. However, it was unclear whether interaction of VAs with mitochondrial targets contributes to the conventional, clinically observable phenotypes of anesthesia and to the collateral phenotypes of exposure to VAs referred to as AiN and AP. Recently, genetic studies have indicated that metabolic interactions may be of more importance than previously appreciated. Because mitochondria are central to both normal cell function (both as energy generators and signaling relays) and stress response, both primary and collateral outcomes from exposure to VAs can result from their mitochondrial effects. Mitochondrial function is shaped by biological (age, genetic background, concurrent inflammation, and degenerative conditions) and environmental factors (stress intensity, duration of exposure to VAs, co-exposure to other agents, and injury) all of which contribute to the varied response to VAs between individuals. When seen within this framework, interaction between VAs and mitochondria can result in a spectrum of outcomes, ranging from anesthesia with rapid, complete recovery to long-term cognitive impairment and from tissue damage to organ protection.

AUTHORS' CONTRIBUTIONS

MP and PGM participated in writing, review, and editing; MMS participated in review and editing; DJ-S contributed in experimental data collection, review, and editing.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: MP: NIGMS R01GM134107 and R&D fund of the Department of Anesthesiology. University of Wisconsin–Madison; PGM: NIGMS R35GM139556 and Northwest Mitochondrial Research Guild; MMS, NIGMS R01GM105696; NIGMS R01GM133865, and Northwest Mitochondrial Research Guild.

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