Common general anesthetic propofol impairs kinesin processivity

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Edited by Michael L. Klein, Temple University, Philadelphia, PA, and approved April 12, 2017 (received for review January 27, 2017)

Propofol is the most widely used i.v. general anesthetic to induce and maintain anesthesia. It is now recognized that this small molecule influences ligand-gated channels, including the GABA_A receptor and others. Specific propofol binding sites have been mapped using photoaffinity ligands and mutagenesis; however, their precise target interaction profiles fail to provide complete mechanistic underpinnings for the anesthetic state. These results suggest that propofol and other common anesthetics, such as etomidate and ketamine, may target additional protein networks of the CNS to contribute to the desired and undesired anesthetic end points. Some evidence for anesthetic interactions with the cytoskeleton exists, but the molecular motors have received no attention as anesthetic targets. We have recently discovered that propofol inhibits conventional kinesin-1 KIF5B and kinesin-2 KIF3AB and KIF3AC, causing a significant reduction in the distances that these processes kinesins can travel. These microtubule-based motors are highly expressed in the CNS and the major anterograde transporters of cargos, such as mitochondria, synaptic vesicle precursors, neurotransmitter receptors, cell signaling and adhesion molecules, and ciliary intraflagellar transport particles. The single-molecule results presented show that the kinesin processive stepping distance decreases 40–60% with EC_50 values <100 mM propofol without an effect on velocity. The lack of a velocity effect suggests that propofol is not binding at the ATP site or allosteric sites that modulate microtubule-activated ATP turnover. Rather, we propose that a transient propofol allosteric site forms when the motor head binds to the microtubule during stepping.

Results

Propofol Decreases the Persistence of MT Gliding but Does Not Alter the Velocity of Movement. To test the hypothesis that propofol can affect kinesin motility, a MT gliding assay was used in conjunction with total internal reflection fluorescence (TIRF) microscopy (26, 27). The advantage of this assay is that it evaluates the ability of the motor population to propel and sustain MT gliding across a lawn of kinesin motors. The first experiment tested the well-characterized homodimeric kinesin-1 K560 (27–29). K560 was bacterially expressed from human kinesin-1 KIF5B, encoding

Significance

Kinesins are major transporters of cargos toward the cell periphery. They are highly expressed in the CNS, and their dysfunction leads to a wide range of human pathologies, including neurodevelopmental and neurodegenerative diseases, ciliopathies, epilepsy, and birth defects. We have discovered that the widely used general anesthetic propofol shortens the distance that kinesins travel, but their velocity remains unchanged. These results suggest that propofol is not binding at the ATP site or allosteric sites that affect ATP turnover, leading to the conclusion that the allosteric sites form on microtubule association. We postulate that general anesthetics bind specifically to transport kinesins and/or the kinesin-%-tubulin interface, and diminish their ability to transport critical cargos, thereby contributing to the pleiotropic state of anesthesia.


The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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3This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1701482114/-/DCSupplemental.

www.pnas.org/cgi/doi/10.1073/pnas.1701482114
the first 560 amino acid residues. A 10-μL perfusion chamber was constructed, and antibodies to the C-terminal His tag of K560 were applied followed by perfusion of MT–K560 complexes. This approach ensures tight binding of the C-terminus of K560 to the coverslip. Subsequently, K560 motility was initiated by 1 mM MgATP. The results in Fig. 1 show that, in the presence of 5 μM propofol, the MTs initially glided across the K560 coverslip as in the DMSO control, but the number of K560-associated MTs decreased dramatically as a function of time as MTs detached from the coverslip and were no longer illuminated in the TIRF field (Movies S1 and S2). Moreover, MT stalling on the coverslip was rarely observed in the presence of propofol. After 5 min, ~80% of MTs glide persistently, but by 20 min, the population remaining on the coverslip was decreased dramatically as a function of time as MTs detached from the DMSO control, but the number of K560-associated MTs decreased to 59.3%. These results were in stark contrast to the DMSO control, where ~95% of the MT population was observed to glide continuously across the K560 coverslip (Fig. 1G).

The MT gliding assay was repeated for kinesin-2 KIF3AC (Fig. 1B, E, and H) and KIF3AB (Fig. 1C, F, and I). These kinesins are distinctive in that they exist physiologically as heterodimers formed from three different gene products: KIF3A, KIF3B, and KIF3C (21–24, 30). The KIF3AC and KIF3AB heterodimers, characterized previously, were engineered to include the N-terminal native motor domain sequence, neck linker, and native helix α7 followed by a dimerization motif to stabilize the native coiled coil (27, 31).

In the absence of propofol, single-molecule experiments show that K560 steps along the MT at a rate of 309.3 nm/s with a run length of 1.03 μm (Fig. 2). Note that, in the presence of 3–5% DMSO, the concentration used as the propofol vehicle, the motility properties of K560, KIF3AC, or KIF3AB were not noticeably altered (Table S1). In contrast, the run length decreased to 0.58 μm in 10 μM propofol, but the velocity was similar at 282 nm/s (Fig. 2A and B and Movies S3 and S4). This run length change is significant, because for processive kinesins, each 8-nm step is coupled to one ATP turnover. Thereby, propofol decreased kinesin processivity from ~129 to 72 steps per run. Subsequent experiments evaluated a propofol concentration dependence, with each data point in Fig. 2C and D representing the average run length and velocity from the Gaussian fit to each histogram dataset as shown for 10 μM propofol in Fig. 1A and B. Fig. 2C shows that the average run length decreased as a function of propofol concentration, with the decrease becoming statistically significant at 5 nM propofol (P < 0.002), but the velocity at each propofol concentration was unaffected. Furthermore, the Hill–Slope model fit to the data provided the EC50 at 58.6 nM.

![Fig. 1. Propofol disrupts the persistence of MT gliding but does not significantly alter the velocity of MT gliding. (A–F) Histograms of velocities (A–C) in control conditions (3% DMSO) and (D–F) at 5 μM propofol for each population of motors. A Gaussian fit provides the average velocity ±SEM for each dataset. The average velocities were not statistically different between DMSO controls and 5 μM propofol datasets (P > 0.5). All experiments were conducted in the presence of 1 mM MgATP. Representative Movies S1 and S2 show K560. (G–I) Persistence of MT gliding as a function of time in 3% DMSO (blue) or 5 μM propofol (red). For all panels, N represents the number of MTs analyzed for each condition. (A, D, and G) Kinesin-1 K560, (B, E, and H) Kinesin-2 KIF3AC, and (C, F, and I) kinesin-2 KIF3AB. (J–M) Anesthetics used in this report: (J) propofol, 2,6-diisopropylphenol; (K) fropfof, 2-fluoro-1,3-diisopropylbenzene; (L) ketamine, 2-(2-chlorophenyl)-2-(methylamino)cyclohexanone; and (M) etomidate, 1-(α-methylbenzyl)imidazole-5-carboxylic acid ethyl ester.](image-url)
Fig. 2. Propofol shortens the mean run length of kinesin-1 K560 but does not alter velocity. (A and B) K560 run length and (Insets) velocity data in (A) 5% DMSO control and (B) 10 μM propofol. Statistical comparison of these data shows that the impact on run length is highly significant \( P < 0.0001 \) but that the effect on velocity is not significant \( P > 0.9 \). All experiments were conducted in the presence of 1 mM MgATP (Movies S3 and S4). (C) Mean run length and (Inset) velocity from K560 single-molecule motility assays plotted as a function of increasing propofol concentration over a range of 0 (5% DMSO control) to 10 μM propofol (log scale). The decrease in run length becomes statistically significant at 5 nM propofol \( P < 0.002 \), whereas the variation in velocity is not statistically significant \( P > 0.5 \). The \( EC_{50} \) was determined from fitting the data to the Hill-Slope model. (D) The fractional inhibition of the run length data at each propofol concentration was plotted as a concentration dependence. The quadratic function provided the \( EC_{50} \) and the maximal fractional inhibition \( I_{\text{max}} \). All values are ±SEM.

To determine the maximal fractional inhibition, the data were analyzed as follows:

\[
I = \frac{\text{Control RL} - \text{Propofol RL}}{\text{Control RL}}
\]

where fractional inhibition, \( I \), is defined as the difference between the run lengths (RL) of the DMSO control and each propofol concentration divided by the control run length (Fig. 2D). The following quadratic equation was fit to the data:

\[
I = \frac{(I_{\text{max}} + P + EC_{50}) - \sqrt{(I_{\text{max}} + P + EC_{50})^2 - 4I_{\text{max}} \cdot P}}{2},
\]

where \( I_{\text{max}} \) is the maximal fractional inhibition, and \( P \) is the propofol concentration. The \( EC_{50} \) here at 61 nM is comparable with the Hill-Slope model estimation at 58.6 nM. The maximal fractional inhibition at 0.46 revealed a significant decrease in K560 run length potential.

**Propofol Also Shortens the Run Length Potential of Kinesin-2 KIF3AC and KIF3AB.** Fig. 3A–D shows the results for the KIF3AC single-molecule studies (Movies S5 and S6). Note that, in the presence of 10 μM propofol, the run length decreased significantly from 1.16 to 0.7 μm \( P < 0.0001 \), but the velocities were unaffected. Like K560, the KIF3AC single-molecule experiments were repeated as a function of propofol concentration (Fig. 3C). The difference in run length became statistically significant at 0.25 nM propofol \( P < 0.0001 \), whereas the velocity remained unchanged. The Hill-Slope model fit to the run length data provided an \( EC_{50} \) at 1.3 nM. Fig. 3D shows the data presented as the fractional inhibition at each propofol concentration. The quadratic fit to these data provided an \( EC_{50} \) at 0.93 nM and the maximal fractional inhibition of 0.40, indicating that propofol shortened the run length potential significantly. Moreover, the \( EC_{50} \) value at ~1 nM is very close to the concentration of the KIF3AC heterodimers in the perfusion chamber at 2 nM Qdot-bound KIF3AC, suggesting the possibility that KIF3A or KIF3C binds propofol more tightly than its partner motor head.

The impact of propofol on KIF3AB was also evaluated (Fig. 3E and F and Movies S7 and S8). The results show that the 0.65-μm run length at 10 μM propofol was significantly less \( P < 0.0001 \) than the run length in the absence of propofol at 1.61 μm. A propofol concentration dependence for KIF3AB was not pursued to determine the \( EC_{50} \) but the 10 μM results clearly show that, as with K560 and KIF3AC, propofol also decreases the run length of KIF3AB considerably \( I_{\text{max}} = 0.60 \) without impacting velocity \( P > 0.2 \).

These results illustrate the remarkable impact that propofol has on the performance of these processive kinesins with \( EC_{50} \) values in the nanomolar range (Fig. 3G). Furthermore, the 40–60% decrease in run length potential suggests a common mechanism, especially based on the overall sequence homology between the catalytic core of the processive kinesin motor domains. Equally intriguing is that each of these processive kinesins could sustain sequential stepping at very high concentrations of propofol and maintain their normal velocity (Fig. 3G).

**Propofol Inhibition Is Dependent on the Propofol Hydroxyl.** Propofol is a fairly simple hydrophobic compound (Fig. 1J), but it contains the 1-hydroxyl that has been linked to molecular recognition within targets that contribute to anesthesia end points. Woll et al. (32) synthesized a compound named fropofol, in which the 1-hydroxyl was substituted with fluoride, dramatically reducing the ability to hydrogen bond (Fig. 1K). This analog maintains a similar molecular volume as propofol with a small increase in hydrophobicity, and fropofol also binds some of the molecular targets of propofol, such as apoferritin (32). With the 1-hydroxyl substitution, fropofol failed to induce loss of mobility end points in Xenopus laevis tadpoles and mice and does not enhance GABAA receptor activity. However, fropofol does retain the propofol-like ability to depress myocardial contractility (8, 32). Therefore, fropofol can be used to separate the desired from some undesired end points of anesthesia. To test the hypothesis that the 1-hydroxyl was necessary for the propofol effect on processive kinesins and thereby, potentially...
attribute this effect to desired end points of anesthesia, we pursued single-molecule experiments with KIF3AC at 10 and 100 μM propofol. Fig. 4 shows that, even at 100 μM propofol, neither the run length of KIF3AC (P > 0.6) nor the KIF3AC velocity (P > 0.3) were significantly diminished. These results clearly show that the propofol 1-hydroxyl is critical for molecular recognition and/or

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kinesin run length inhibition and that kinesins may contribute to propofol-induced unconsciousness as reflected by loss of mobility end points.

**Propofol Shortens the Run Length Potential of Homodimeric KIF3BB and KIF3CC, but No Effect Is Observed for KIF3AA.** We were intrigued by the EC50 at ∼1 nM propofol for KIF3AC, because the single-molecule experiments were performed at 2 nM KIF3AC dimer. We questioned whether this constant may reflect a difference in propofol binding affinity to KIF3A compared with KIF3C. To explore this hypothesis further, single-molecule experiments at 10 μM propofol were pursued with engineered homodimers of KIF3AA, KIF3BB, and KIF3CC (Fig. 5). The homodimer design was similar to that of the heterodimers, where each polypeptide included the native catalytic motor domain, the neck linker, helix α7 followed by a dimerization domain, the Tobacco Etch Virus site, and the His8 tag (27). The results were surprising. Although 10 μM propofol shortened the run length potentials of homodimeric KIF3BB (I_max = 0.56) and KIF3CC (I_max = 0.38), propofol seemed to have negligible effect on KIF3AA (I_max = 0.02).

When the sequences of the motor domain were compared, KIF3B and KIF3C show 71% identity, but the identity between KIF3A and KIF3B or KIF3C is less at 69 and 57%, respectively. Kinesin-1 KIF5B was clearly affected by propofol, but its sequence identity compared with KIF3A, KIF3B, and KIF3C is less than 50%. However, structurally kinesins share a highly conserved Walker nucleotide binding fold that consists of a central twisted β-sheet and three nucleotide binding loops designated switch-1, switch-2, and the P loop (33–37). Kinesins also share a similar MT binding interface and a series of structural transitions in response to the nucleotide binding state that coordinates MT association and detachment (38–41). To sustain a processive run, the domains must be coordinated, so that one is always in a MT strongly bound state to prevent motor detachment from the MT. Therefore, these results suggest that, despite the relatively high homology between these motor domains, small sequence differences in the motor domain have resulted in KIF3A either not binding propofol and/or propofol not promoting premature motor detachment from the MT.

**Etomidate and Ketamine Also Inhibit the Run Length Potential of KIF3AC.** General anesthetics have multiple functional targets and overlapping binding sites within their target proteins, in part because they are small hydrophobic molecules. To determine if processive kinesins are affected by other i.v. general anesthetics, we tested whether kinesin motility of KIF3AC was altered by ketamine or etomidate (Fig. 1L and M). Etomidate, like propofol, enhances the GABAA-mediated inhibitory response, whereas ketamine acts primarily as an antagonist of NMDA receptors, although it too has multiple targets, including a subset of the G protein (heterotrimeric

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**Fig. 5.** Propofol does not affect homodimeric KIF3AA single-molecule motility. (A–F) Single-molecule run length and (Insets) velocity data for (A and B) KIF3AA, (C and D) KIF3BB, and (E and F) KIF3CC comparing control conditions at (A, C, and E) 5% DMSO vs. (B, D, and F) 10 μM propofol. All experiments were conducted at 1 mM MgATP. (G) Compiled single-molecule motility data. I_max is calculated based on the fractional inhibition at 10 μM propofol.
guanine nucleotide binding protein)-coupled receptors that are distributed throughout the CNS (10–14). The results in Fig. S1 show that, like propofol, 10 μM etomidate or ketamine shortened the run length potential of kinesin-2 KIF3AC: from 1.19 to 0.57 μm by etomidate and from 1.19 to 0.52 μm by ketamine (P < 0.0001). Also, like propofol, neither drug altered the velocity of movement significantly (P > 0.7). These results implicate processive kinesins as anesthetic targets that may contribute to modulation of the anesthetic state.

Discussion

There Is a Common Mechanism to Shorten Run Length Potential. Because propofol did not alter the velocity of movement for K560, KIF3AC, or KIF3AB in the MT gliding or single-molecule experiments, we propose that propofol is not binding at allosteric sites within the catalytic motor domain that would alter MT-activated ATP turnover. Moreover, because the concentration of MTs is at 0.1 μM in the single-molecule assays and the EC50 values are <100 nM propofol, it is unlikely that propofol is saturating binding sites along the MT where the kinesin head would step during a processive run. Moreover, the lack of a propofol effect on KIF3AA renders a common MT site unlikely. Rather, these results indicate that propofol and likely, etomidate and ketamine allosteric binding sites form transiently in kinesin when the motor domain binds to the MT lattice during stepping, reducing kinesin MT affinity. This outcome, in essence, implies a druggable allosteric site that, when occupied, promotes detachment of the kinesin motor from the MT. There may exist multiple transient propofol and therefore, etomidate and ketamine binding sites on the kinesin motor domain, requiring only anesthetic binding to kinesin to impact the motor’s interactions with the MT. Note too that the maximal fractional inhibition promoted by propofol, etomidate, and ketamine was 0.4–0.6, suggesting a common mechanism for shortening the run length of these kinesins. This inhibition profile is very different from the loop L5-targeting small molecules in either the monastral family of inhibitors for human kinesin-5 KSP/Eg5 (45–48) or the kinesin-specific inhibitor GSK923295 for kinesin-7 CENP-E (49). Monastral family inhibitors stabilize ADP at the active site and therefore, destabilize the MT–KSP interaction. GSK923295 inhibits the release of inorganic phosphate and stabilizes the interaction of CENP-E with the MT. However, both inhibitors alter MT-activated ATPase activity as well as kinesin-promoted motility, which is very different from the results presented here.

One can reason based on kinesin X-ray crystal structures and site-specific mutations that small, hydrophobic anesthetic molecules have the potential to bind at multiple allosteric sites on the kinesin motor domain or at residues of the MT; kinesin interface to alter kinesin binding affinity to the MT and promote motor detachment (38, 39, 50–54).

Are Processive Kinesins Targets of General Anesthetics, and Thereby, Do They Contribute to the Anesthetic State? The binding of general anesthetics to ligand- or voltage-gated channels and receptors is known to elicit both desired and undesired end points of anesthesia. However, it is also recognized that none of these molecular targets have satisfied the criteria of being both necessary and sufficient to produce the complete anesthetic state. Other plausible targets have included mitochondria, tubulin, and the synaptic vesicle transport and release machinery (17, 55–58). For example, SNAP-25 and syntaxin specifically bind propofol (7). Therefore, it is reasonable that there are other targets that interact with anesthetics and contribute to the state of anesthesia. Our results expand the field by providing evidence for a largely overlooked but critically positioned set of targets, molecular motors, which could influence acute and/or chronic end points observed with some general anesthetics.

The EC50 values for kinesin “derailing” are at least 10-fold lower than those associated with propofol-induced immobility, suggesting that these effects may underlie other subclinical actions of propofol, such as amnesia and postural instability. Alternatively, it is possible that EC50 values will be very different in the crowded intracellular milieu and when the motors are loaded with cargo. Nevertheless, these results clearly indicate that kinesin motors will be influenced during propofol anesthesia in vivo, and it seems unlikely that such important intracellular movers will not contribute to components of anesthetic action.

The human kinesin superfamily includes 45 genes, 38 of which are expressed in brain, with three subfamilies of kinesins that are predominantly responsible for cargo transport to the cell periphery (i.e., the synapse in neurons) (21, 22, 24, 25, 30, 59, 60). Kinesins are the major anterograde transporters of cargos that have been established as anesthetic targets, including mitochondria, GABA_A receptors, syntaxin, and SNAP-25 (7, 9, 57, 58, 61–64). The critical neurological role of kinesins is further indicated by the lethality of many mutations, an anesthetic-like immobility phenotype in others, and numerous kinesin dysfunctions linked to a wide range of human pathologies, including neurodevelopmental and neurodegenerative diseases, ciliopathies, epilepsy, and birth defects (21, 24, 64–67).

In summary, the flux of cellular substrates is a balance between the collective anterograde transport by kinesins and retrograde transport by dynein. Even a modest depletion of kinesin-1 or -2 processivity would create an opportunity for retrograde motors, like dynein, to drive cargo transport back to the cell body, leading to a critical imbalance of cargo distribution. Therefore, kinesins are critically positioned to underlie specific anesthesia end points, and this report has revealed a dramatic impact of three different anesthetics on processive kinesins at concentrations used in routine clinical care.

Methods

Standard MT gliding and single-molecule kinesin Qdot assays and TIRF imaging techniques were used throughout. Detailed descriptions of kinesin motor construct design, expression, and purification and microscopy methods are provided in SI Methods. MT concentrations are reported as paclitaxel-stabilized α,β-tubulin concentration.

ACKNOWLEDGMENTS. We thank Pei Tang (Department of Anesthesiology, University of Pittsburgh) for her initial suggestion that propofol might affect kinesin motility and Nicole Stoddard, who initiated this project as her Rensselaer Senior Research Thesis. This work was supported, in part, by an award from the Rensselaer Office of Research, National Science Foundation Graduate Research Fellowship Program Grant DGE-1321851 (to K.A.W.), and NIH Grants P01-GM58576 (to R.G.E.) and R37-GM054141 (to S.P.G.).


SI Methods

Kinesin-1 K560 Homodimers. Human kinesin-1 K560 was expressed from Addgene plasmid K560HTR 24444, which encodes the first 560 amino acid residues of KIF5B followed by a C-terminal His$_{6}$ tag (28). The plasmid was transformed into E. coli CodonPlus (DE3)-RIL cells (Stratagene Corp.) with selection on LB plates containing 100 μg/mL ampicillin and 10 μg/mL chloramphenicol. Positive clones were selected and grown in LB medium with antibiotics at 37 °C until the cultures reached an $A_{600}$ of ~0.35. The temperature was shifted to 16 °C on ice, and the cultures were subsequently induced at 0.1 mM isopropyl-1-thio-β-D-galactopyranoside followed by incubation with shaking for 16–18 h at 16 °C. Cell pellets were collected by centrifugation and resuspended at 5 mL lysis buffer per g cells at 4 °C. Lysis buffer contained 20 mM sodium phosphate buffer, pH 6.9, 100 mM NaCl, 2 mM MgCl$_{2}$, 0.1 mM EGTA, 0.05 mM ATP, 1 mM DTT, and 5 mM PMSF. One SIGMAFAST Protease Inhibitor Mixture Tablett (Sigma-Aldrich) was added to the resuspended cells followed by adjusting to 1 mM Mg$_{2}$$^{+}$ lysosome (Sigma-Aldrich). The cells were stirred in an ice bath at 4 °C for 60 min and then lysed by three cycles of freeze (liquid N$_{2}$) and thaw (37 °C water bath with tube inversion). Cell lysates were clarified by ultracentrifugation, and the supernatant was allowed to equilibrate to room temperature.

The supernatant enriched in K560 was adjusted to 0.1 mM GTP, 40 μM paclitaxel, 5 μM MT, and 0.1 mM Adenosine 5′-(β,γ-imido) triphosphate (AMPPNP) followed by incubation at room temperature for 20 min to promote MT+$\times$K560 complex formation. After centrifugation (38,000 g for 35 min at 25 °C), the supernatant was discarded. The MT–K560 pellet was resuspended in 5 mL of lysis buffer without PMSF; adjusted to 1 mM MgATP, 150 mM KCl, and 40 μM PMSF, and incubated for 20 min to release active kinesin. The supernatant, the K560-enriched supernatant was transferred to a fresh tube and adjusted to 300 mM NaCl plus 20 mM sodium phosphate buffer, pH 6.9.

The K560 supernatant was loaded onto a HiTrap FF Ni$_{2+}$-NTA column (GE Healthcare), which was equilibrated in Ni$_{2+}$-NTA Binding Buffer at 4 °C (20 mM sodium phosphate buffer, pH 6.9, 300 mM NaCl, 2 mM MgCl$_{2}$, 0.1 mM EGTA, 1 mM DTT, 0.02 mM ATP, 20 mM imidazole). The column was washed until the absorbance returned to baseline. K560 was eluted using a linear gradient of 20–400 mM imidazole, pH 6.9, in Ni$_{2+}$-NTA Binding Buffer. K560-enriched fractions were identified by SDS/PAGE, pooled, and dialyzed overnight against dialysis buffer (20 mM Hepes, pH 7.2, 250 mM NaCl, 0.1 mM EGTA, 0.1 mM EDTA, 5 mM magnesium acetate, 50 mM potassium acetate, 1 mM DTT). The dialysis buffer was changed to 20 mM Hepes, pH 7.2, 250 mM NaCl, 0.1 mM EDTA, 0.1 mM EGTA, 5 mM magnesium acetate, 50 mM potassium acetate, and 1 mM DTT plus 5% sucrose, and the K560 was dialyzed for an additional 2 h followed by ultracentrifugation to remove protein aggregates. The supernatant was concentrated, aliquoted, and flash frozen in liquid nitrogen before storage at ~80 °C. Based on SDS/PAGE analysis, this purification scheme resulted in high-purity protein without fragmentation. The predicted molecular weight at 129,114.

Kinesin-2 KIF3 Heterodimeric and Homodimeric Motors. The Mus musculus KIF3A, KIF3B, and KIF3C plasmids for expression of the KIF3AB (31) and KIF3AC (27) heterodimers as well as homodimers of KIF3AA, KIF3BB, and KIF3CC were described previously in detail along with their expression and purification (27).

The construct for KIF3A when expressed as a heterodimer with KIF3C includes the native sequence of the motor domain, the neck linker, and helix $\alpha 7$ (M1–L374) followed by the dimerization motif of EB1 (bold), the C-terminal Tobacco Etch Virus (TEV) protease-cleavable site (italics), linker (plain font) and StrepII tag (underlined): KIF3A(M1–L374)-DFYFGKLRNIELICQENEGENDPVLRQIDILYATDTESSNYFGASWNWHPQFEK. The KIF3C construct for KIF3AC includes the native sequence of the KIF3C motor domain, the neck linker, and helix $\alpha 7$ (M1–L374) followed by the dimerization motif of EB1 (bold), the TEV protease-cleavable site (italics), and His$_{6}$ tag (underlined) with the linker residues in plain font: KIF3C(M1–L374)-DFYFGKLRNIELICQENEGENDPVLRQIDILYATDTESSNYFGASHHHHHHHHHHH.

To generate a stable heterodimer of KIF3AB, a synthetic heterodimerization domain motif containing either an acidic heterodimerization domain (AHD) or a basic fusion helix heterodimerization domain (BHD) was used (31, 68, 69). The KIF3A-AHD polypeptide consisted of the KIF3A motor domain, the neck linker, and three heptads of native helix sequence followed by the AHD helix (bold), a TEV protease site (italics), linker residues (plain font), and StrepII tag (underlined): KIF3A(M1–E376)-LEKEIALEKAAILKTESSNYFGASWNWHPQFEK. The KIF3B-BHD polypeptide contained the KIF3B motor domain, the neck linker, and three heptads of native helix sequence followed by the BHD helix (bold) and a TEV protease site (italics) with linker residues (plain font) and a His$_{6}$ tag (underlined): KIF3B(M1–K371)-LEKEIALEKAAILKTESSNYFGASHHHHHHHHHHHHH. For the KIF3AA, KIF3BB, and KIF3CC homodimers, each native N-terminal motor domain sequence, neck linker, and $\alpha 7$ helix was C-terminally fused to an in-register segment of the dimerization motif from EB1 (bold) followed by the TEV protease site (italics) with linker residues (plain font) and a His$_{6}$ tag (underlined): KIF3A: KIF3A(Met$_{1}$–Leu$_{8}$)-DFYFGKLRNIELICQENEGENDPVLRQIDILYATDTESSNYFGASHHHHHHHHHHHHHNNB: KIF3B:KIF3B(Met$_{1}$–Leu$_{8}$)-DFYFGKLRNIELICQENEGENDPVLRQIDILYATDTESSNYFGASHHHHHHHHHHHHH; KIF3C: KIF3C(M1–L374)-DFYFGKLRNIELICQENEGENDPVLRQIDILYATDTESSNYFGASHHHHHHHHHHHHHH.

Note that the EB1 motif is a dimerization domain only and does not interact with MTs as shown previously (27, 70, 71). For all of the experiments reported here, the TEV-cleavable purification tags were left intact.

KIF3 Protein Expression and Purification. All KIF3 motors were expressed in the E. coli BL21-CodonPlus (DE3)-RIL cell line (Stratagene) with heterodimers resulting from cotransformation of two plasmids and selection on LB plates containing 100 μg/mL ampicillin, 50 μg/mL kanamycin, and 10 μg/mL chloramphenicol (27, 31). To achieve the purification of KIF3AC and KIF3AB, sequential affinity columns were used. The HiTrap FF Ni$_{2+}$-NTA column (GE Healthcare) first selected for the C-terminally His$_{6}$-tagged KIF3B or KIF3C followed by the StrepTactin column (GE Healthcare), which selected for the Strep-tagged KIF3A. The purified KIF3AC and KIF3AB motors were evaluated by analytical gel filtration chromatography (Superose 10;300; GE Healthcare) and SDS/PAGE to confirm purification of stable heterodimers with a 1:1 stoichiometry of KIF3A to either KIF3C or KIF3B. The predicted molecular weight based on amino acid sequence of KIF3AC is 98,317, and the predicted molecular weight based on amino acid sequence of KIF3AB is 92,131.
The KIF3 proteins were dialyzed against ATPase buffer containing 20 mM Hepes, pH 7.2, with KOH, 5 mM magnesium acetate, 0.1 mM EDTA, 0.1 mM EGTA, 50 mM potassium acetate, and 1 mM DTT plus 5% sucrose followed by ultracentrifugation to remove aggregates and then frozen as aliquots in liquid N2 for storage at −80 °C.

To purify homodimeric KIF3AA, KIF3BB, or KIF3CC, the supernatant of the cell lysate was loaded onto the HisTrap FF Ni²⁺-NTA column (GE Healthcare) to select for the His₈ tag followed by additional purification using gel filtration on an HPLC Superose 10/300 gel filtration column (GE Healthcare) with elution into the 20 mM Hepes dialysis buffer described above plus 200 mM NaCl. The purity of the KIF3AA, KIF3BB, or KIF3CC homodimers was subsequently analyzed by analytical gel filtration and SDS/PAGE. The predicted molecular weight based on amino acid sequence of KIF3AA is 97,000, the predicted molecular weight based on amino acid sequence of KIF3BB is 96,022, and the predicted molecular weight based on amino acid sequence of KIF3CC is 99,518.

Before each experiment, KIF3 protein aliquots were thawed and clarified for 10 min at 4 °C (313,000 × g; TLA-100 rotor; Beckman Coulter TLX Optima Ultracentrifuge), and the protein concentration was determined using the Bio-Rad protein assay with IgG as a protein standard.

### In Vitro MT Gliding Assays

Polarity marked X-rodamine-labeled MTs were polymerized as described (26, 27, 69) and stabilized with 20 μM paclitaxel. MT motor complexes were performed with a final concentration of 0.5 μM tubulin (paclitaxel-stabilized α,β-tubulin) and 2.5 μM kinesin dimer. Perfusion chambers were constructed using acid-washed coverslips and incubated first with 40 μg/mL Penta-His antibodies (Qiagen) for 10 min followed by a 5-min incubation of blocking buffer (PME80; 1.5 mM magnesium acetate, 500 μg/mL casein, 25 mM glucose, 0.2 mg/mL glucose oxidase, 175 μg/mL catalase, 0.3 mg/mL creatine phosphokinase, 2 mM phosphocreatine, 0.5% β-mercaptoethanol, 15 μM paclitaxel). MT motor complexes were then introduced into the chamber and allowed to bind for 5 min. Unbound complexes were removed with excess blocking buffer. Gliding via the surface-bound motors was activated by introducing Activity Buffer (blocking buffer supplemented with 1 mM MgATP and either 3% DMSO or 5% propofol) to give a final concentration of 2 nM ATPase buffer activated motor activity, and chambers were imaged immediately.

**TIRF Microscopy and Image Acquisition.** Chambers were imaged by TIRF microscopy at 25 °C using a Zeiss Inverted Axio Observer Z1 MOT fluorescence microscope with the 100x oil 1.46 N.A. Plan-Apochromat objective (Carl Zeiss Microscopy, Inc.) and an incubation hood as described (27). Digital images were collected through a Hamamatsu electron multiplier EM-CCD digital camera using the AxioVision 4.8.2 software package. This imaging method yielded 512 × 512-pixel images with 0.16 μm per pixel in both x and y planes. The Qdot complexes were tracked by imaging at 488 nm (5% laser power) every ~0.5 s for 5 min using 100-ms exposure. Reference images of the X-rodamine MT tracks were taken at 564 nm (2% laser power) with 300-ms exposure both before and after acquisition of the Qdot channel. Qdot images were then overlaid with the MT image using NIH ImageJ software.

**Data Analysis.** Single-molecule Qdot motility was analyzed using the MultipleKymograph plugin for ImageJ (J. Rietdorf and A. Seitz, European Molecular Biology Laboratory, Heidelberg, Germany). Velocity histograms were plotted, and a Gaussian function was applied to determine the mean velocity ±SEM. Run lengths were plotted as histograms using a single-exponential decay fit to the data to determine mean run length:

\[ y = y_0 + A \left( \frac{1}{l} \right) \]

where \( A \) is the maximum amplitude, and \( l \) is the mean run length reported ±SEM. The first bin of run-length histograms was masked from the fit because of the resolution limit of the TIRF microscope (<0.25 μm). Statistical analyses for run lengths and velocities were performed using the StatPlus plugin for Microsoft Excel (AnalytSoft Inc.). The Comparing Means (t test assuming different variances) algorithm with an α-reliability level of 5% was used for both parameters, with velocities compared with a two-tailed \( t \) test, whereas run lengths were compared with a one-tailed \( t \) test.

To ensure that the maximum run length potential of the Qdot–motor complexes was visualized, Qdots were excluded from the analysis if they fell off at the end of the MT, paused at the MT end, or began or ended a run outside the timescale of the experiment. Only long MT tracks were examined to allow...
for the collection of both long and short runs from the same MT tracks, thereby avoiding data bias. Pausing and stalling occurred for all motors tested in both the absence and presence of propofol but represented only a minor fraction (2–6%) of each dataset and showed no apparent correlation between pause frequency or duration and propofol concentration. The data that included pausing events were eliminated from additional analysis.

Fig. S1. The general anesthetics ketamine and etomidate decrease the run length potential of KIF3AC (related to Fig. 3). (A–C) Run length and (Inset) velocity data collected for KIF3AC in the presence of (A) 5% DMSO control, (B) 10 μM ketamine•HCl 5-(+)2-(2-chlorophenyl)-2-(methylamino)cyclohexanone hydrochloride, and (C) 10 μM etomidate, (R)-1-(α-methylbenzyl)imidazole-5-carboxylic acid ethyl ester. The difference in run length is statistically significant (P < 0.0001) between the control and ketamine or etomidate, whereas the difference in velocity showed no statistical significance (P > 0.7).

<table>
<thead>
<tr>
<th>Kinesin</th>
<th>Velocity (nm/s)</th>
<th>Run length (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5% DMSO</td>
<td></td>
</tr>
<tr>
<td>K560</td>
<td>305.1 ± 5.2</td>
<td>309.3 ± 9.9</td>
</tr>
<tr>
<td>KIF3AC</td>
<td>168.6 ± 5.6</td>
<td>182.1 ± 5.4</td>
</tr>
<tr>
<td>KIF3AB</td>
<td>246.2 ± 11.0</td>
<td>207.8 ± 2.3</td>
</tr>
<tr>
<td>KIF3AA</td>
<td>239.2 ± 2.2</td>
<td>286.1 ± 7.7</td>
</tr>
<tr>
<td>KIF3BB</td>
<td>327.6 ± 7.2</td>
<td>281.5 ± 11.0</td>
</tr>
<tr>
<td>KIF3CC</td>
<td>8.2 ± 0.3</td>
<td>6.9 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>—*</td>
<td>—*</td>
</tr>
<tr>
<td>K560</td>
<td>1.26 ± 0.10</td>
<td>1.03 ± 0.10</td>
</tr>
<tr>
<td>KIF3AC</td>
<td>1.23 ± 0.09</td>
<td>1.16 ± 0.05</td>
</tr>
<tr>
<td>KIF3AB</td>
<td>1.62 ± 0.11</td>
<td>1.61 ± 0.33</td>
</tr>
<tr>
<td>KIF3AA</td>
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</tr>
<tr>
<td>KIF3BB</td>
<td>1.51 ± 0.16</td>
<td>1.29 ± 0.17</td>
</tr>
<tr>
<td>KIF3CC</td>
<td>0.57 ± 0.03</td>
<td>0.79 ± 0.07</td>
</tr>
</tbody>
</table>

*Data in the absence of DMSO were reported previously (27).
Movie S1. K560-driven MT gliding control in conditions with 3% DMSO, 1 mM MgATP, and 2.5 μM K560 dimer. Average gliding velocity was 361.6 ± 5.6 nm/s, with continuous gliding throughout the video. Video playback speed is at 60× real time.

Movie S1

Movie S2. K560-driven MT gliding in the presence of 5 μM propofol. Chamber conditions also include 1 mM MgATP and 2.5 μM K560 dimer. Average gliding velocity was 383.0 ± 5.3 nm/s, with a significant propofol-induced MT loss from the gliding surface as a function of time. Video playback speed is at 60× real time.

Movie S2
Movie S3. Single-molecule motility control of 2 nM K560 with 5% DMSO and 1 mM MgATP. Average velocity was 309.3 ± 9.9 nm/s, and mean run length was 1.03 μm. Video playback speed is at 20× real time.

Movie S3

Movie S4. Single-molecule motility of 2 nM K560 at 10 μM propofol and 1 mM MgATP. Average velocity is similar to the control at 282.0 ± 17.1 nm/s, whereas mean run length of the population decreases significantly to 0.58 ± 0.03 μm. Video playback speed is at 20× real time.

Movie S4
Movie S5. Single-molecule motility control of 2 nM K13AC with 5% DMSO and 1 mM MgATP. The average velocity is 182.1 ± 6.2 nm/s, and mean run length is 1.16 ± 0.05 μm. Video playback speed is at 20x real time.

Movie S5

Movie S6. Single-molecule motility of 2 nM K13AC at 10 μM propofol and 1 mM MgATP. Average velocity is unchanged from the control at 183.6 ± 6.2 nm/s, whereas mean run length of the population is significantly decreased to 0.7 ± 0.03 μm. Video playback speed is at 20x real time.

Movie S6
**Movie S7.** Single-molecule motility control of 2 nM KIF3AB at 5% DMSO and 1 mM MgATP. Average velocity is 207.8 ± 2.3 nm/s, and mean run length is 1.61 ± 0.33 μm. Video playback speed is at 20× real time.

**Movie S8.** Single-molecule motility of 2 nM KIF3AB at 10 μM propofol and 1 mM MgATP. Average velocity remains similar to the control at 173.9 ± 7.1 nm/s, whereas mean run length of the population is significantly decreased to 0.65 ± 0.05 μm. Video playback speed is at 20× real time.