Thalamic Microinfusion of Antibody to a Voltage-gated Potassium Channel Restores Consciousness during Anesthesia

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Background: The Drosophila Shaker mutant fruit-fly, with its malfunctioning voltage-gated potassium channel, exhibits anesthetic requirements that are more than twice normal. Shaker mutants with an abnormal Kv1.2 channel also demonstrate significantly reduced sleep. Given the important role the thalamus plays in both sleep and arousal, the authors investigated whether localized central medial thalamic (CMT) microinfusion of an antibody designed to block the pore of the Kv1.2 channel might awaken anesthetized rats.

Methods: Male Sprague-Dawley rats were implanted with a cannula aimed at the CMT or lateral thalamus. One week later, unconsciousness was induced with either desflurane (3.6 ± 0.2%; n = 55) or sevoflurane (1.2 ± 0.1%; n = 51). Arousal effects of a single 0.5 μl infusion of Kv1.2 potassium channel blocking antibody (0.1–0.2 mg/ml) or a control infusion of Arc-protein antibody (0.2 mg/ml) were then determined.

Results: The Kv1.2 antibody, but not the control antibody, temporarily restored consciousness in 17% of all animals and in 75% of those animals where infusions occurred within the CMT (P < 0.01 for each anesthetic). Lateral thalamic infusions showed no effects. Consciousness returned on average (± SD) 170 ± 99 s after infusion and lasted a median time of 398 s (interquartile range: 279–510 s). Temporary seizures, without apparent consciousness, predominated in 35% of all animals.

Conclusions: These findings support the idea that the CMT plays a role in modulating levels of arousal during anesthesia and further suggest that voltage-gated potassium channels in the CMT may contribute to regulating arousal or may even be relevant targets of anesthetic action.

POTASSIUM (K) channels play a major role in regulating tissue excitability. There are at least four different types of K channels that serve slightly different functions in the nervous system, including voltage-gated (Kv), calcium-activated (KCa), inward rectifying (Kir), and 2-pore (K2P) domain background leak channels. Many authors have suggested that K channels are involved in producing the effects of anesthesia. Much recent focus has been placed on distinguishing K2P channels as possible targets of anesthetic action, yet interactions with various other K channels like the Kv channels may also be important.

Kv channels are further divided into 12 families (Kv1–Kv12) on the basis of sequence homology and similarity to Drosophila melanogaster (i.e., fruit-fly) genes. In Drosophila, the Kv channel genes produce multiple versions of a particular channel and are more commonly known by historical nomenclature such as: Shaker (Kv1.1–Kv1.8), Shab (Kv2.1–Kv2.2), Shaw (Kv3.1–Kv3.4), Shal (Kv4.1–Kv4.3), and eberago-go (Kv10.1–Kv10.2). The eight members of the Shaker-related K channel family (Kv1.1–Kv1.8) are involved with generating voltage-dependent outward currents that regulate action potential threshold, as well as waveform and pacemaker activity in excitable tissue. Kv channels are generally composed of tetramers of alpha subunits. When they are expressed as homomeric channels, most have “delayed rectifier” properties, and the others will exhibit fairly rapid inactivation. The different Kv.x family members (where x is any one of the possible eight different subunits) can coassemble into channels with mixed heteromeric alpha subunit compositions. Furthermore, Kv-beta subunits also exist and add on another layer of complex functional diversity in vivo. The subunit composition of the Kv1 channels not only determines their gating and kinetic properties, it also dramatically affects their expression and localization.

The idea that Kv channels might play a role in anesthesia emerged from the discovery that Drosophila Shaker mutants shake their legs vigorously during ether anesthesia. The Shaker mutant lacks a normal functioning Kv1.x channel, suggesting that the suppression of neural activity under ether anesthesia depends to some extent on a properly functioning Kv1.x channel. Indeed, the amount of isoflurane needed to anesthetize a Shaker mutant with a completely nonfunctioning Kv1.x channel is more than twice the dose needed to anesthetize wild-type flies. Importantly, the changes in isoflurane doses needed to anesthetize various other Shaker mutants parallels the expected reductions in ionic currents mediated through the respective malfunctioning K channels. In other words, the more defective the K channel (and the less current that passes through it), the

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greater the dose of isoflurane needed to anesthetize a particular *Shaker* mutant. This seems to suggest that anesthesia might work in part by hijacking the functioning of the Kv1.x channel.

Recently, the unconsciousness of sleep was also linked to a voltage-dependent K channel.\(^\text{15}\) Mutagenesis analysis was used to screen more than 9,000 *Drosophila* lines to identify those with a limited ability to sleep. Genetic analysis of these flies revealed a point mutation in a conserved domain of the *Shaker* gene that involves the voltage sensing portion of Kv1.2 channel.\(^\text{15,16}\) Thus, for *Drosophila* to sleep, it appears to need current to properly flow through its Kv1.2 channels. Taken together with the earlier anesthesia work, this suggests Kv1.2 channels might be involved with mediating the effects of volatile anesthetics on consciousness.

Concurrent with the developments in sleep neurophysiology, a possible role for the central medial thalamus (CMT) in contributing to the unconsciousness of anesthesia was recently identified.\(^\text{17}\) Anesthetic-induced unconsciousness can be reversed with a localized and site-specific microinfusion of nicotine into the CMT of rats.\(^\text{17}\) As the loss of consciousness associated with sleep and anesthesia may share overlapping neurobiological mechanisms,\(^\text{18–21}\) and nicotine is known to also block various K channels,\(^\text{22,23}\) including some Kv channels,\(^\text{24}\) the hypothesis is raised that anesthetic effects on consciousness might involve interactions with Kv1.2 channels located, in part, in the CMT. Herein, we open the investigation into this area of research by microinfusing a Kv1.2 channel blocking antibody directly into the CMT of rats placed in an anesthetic chamber exposed to a dose of inhalational agent that is just sufficient to render them unconscious. As in the case of *Drosophila Shaker* mutants, which have a chronic malfunction of Kv1.x channels, the acute conduction blockade of the Kv1.2 channels in the CMT should act to rapidly increase the anesthetic dose required to keep the animals unconscious. As the dose of anesthesia will be held constant after the localized antibody microinfusion, a positive result (indicating the possible contribution of Kv1.2 channels to inducing the unconsciousness of anesthesia) will be manifest as a behavioral arousal of the animals; that is, they should awaken in the chamber filled with anesthesia.

### Materials and Methods

All research activities were conducted with full approval of the Institutional Animal Care and Use Committee of the University of California, Irvine.

#### Animals

A total of 106 Sprague-Dawley rats (250–280 g or approximately 9 weeks old on arrival) were obtained from Charles River Laboratories, Inc. (Wilmington, MA). They were housed individually in a temperature-controlled (22°C) colony room, with food and water available *ad libitum*. Animals were maintained on a 12-h light, 12-h dark cycle (0700–1900 lights on).

#### Surgery

Rats were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal) and placed into a stereotaxic frame (Benchmark Digital Stereotaxic, Saint Louis, MO). A guide cannula (23-gauge) was placed, aimed at the central medial thalamus (coordinates: anteroposterior -3.0 mm; mediolateral +1.7 mm, with 13-degree tilt; dorsoventral -4.5 mm; incisor bar, -3.3 mm). The guide cannula was 2 mm shorter in length than needed to reach the central medial thalamus. Indeed, the end of the guide cannula did not reach into the thalamus proper. The thalamus was entered only at the time of the experiments when the microinfusion was delivered through a microinfusion needle inserted into the guide cannula that was 2 mm longer than the guide cannula itself. For the animals given desflurane anesthesia (*n* = 55), all cannulae targeted the CMT. For the animals given sevoflurane (*n* = 51), most targeted the CMT, but ten animals were used as location controls; five targeted the ventral lateral thalamic nucleus (coordinates: anteroposterior -3.0 mm; mediolateral +1.7 mm; dorsoventral -4.5 mm), and five targeted the posterior thalamic nucleus (coordinates: anteroposterior -3.0 mm; mediolateral +1.7 mm; dorsoventral -3.5 mm). Dental acrylic and skull screws secured each cannula. Animals were allowed 6–7 days to recover before experiments.

#### Drugs

The Kv1.2 antibody was a gift from Chiara Cirelli, M.D., Ph.D. (Associate Professor, Department of Psychiatry, University of Wisconsin, Madison, Wisconsin) and Giulio Tononi, M.D., Ph.D. (Professor, Department of Psychiatry, University of Wisconsin, Madison, Wisconsin). Kv1.2 rabbit polyclonal antibodies were made and affinity-purified through a contracted manufacturer (Genemed Synthesis Inc, San Francisco, CA), during performance of a grant with the Defense Advanced Research Projects Agency. The antibody was manufactured by following the specifications of Zhou *et al.*\(^\text{25}\) Zhou *et al.* generated specific antipeptide antibodies to epitopes in the external vestibule of the Kv1.2 delayed-rectifier potassium channel. Their antibody was found to block 70% of the whole-cell Kv1.2 currents in transfected cells in a concentration and time-dependent manner.\(^\text{25}\) Specificity was established by showing that the antibody did not block currents to Kv1.3 or Kv3.1 channels, and binding was mutually exclusive with α-dendrotoxin,\(^\text{25}\) a channel blocker that also binds to the external vestibule of the Kv1.2 channel.\(^\text{30}\) In the current work, the antibody was diluted in normal saline immediately before...
infusion into the thalamus of anesthetized rats. Initial infusions were performed with a concentration of 0.2 mg/ml antibody in 0.5-μl infusion volume given over 1 min. A large proportion of seizure responses prompted the lowering of the dose used to 0.1 mg/ml antibody in the same 0.5-μl infusion volume.

As antibodies are relatively large molecules (approximately 150 kDa), the in vivo use of an antibody infusion given directly into a discrete region of the brain might cause some type of nonspecific dysfunction to occur. Whereas many examples of using antibodies as in vivo probes for specific receptor-targets now exist, we nevertheless controlled for the possibility that a nonspecific arousal effect might occur due to the infusion of an antibody itself. To evaluate this possibility, we infused nine animals under sevoflurane anesthesia with an antibody directed against an intracellular nonreceptor target, the activity regulated cytoskeletal (Arc) protein. This Arc rabbit polyclonal antibody was purchased from a commercial vendor (BioVision Research Products, Mountain View, CA). We injected 0.2 mg/ml Arc antibody in phosphate-buffered saline given in a single 0.5-μl microinfusion.

Consciousness Suppression with Anesthesia
After recovery from cannula implantation (after 6–7 days), animals were anesthetized in a clear chamber as previously described. Briefly, animals were placed in a rectangular 84 clear Plexiglas anesthetizing chamber and exposed to anesthesia in air at 2 l · min⁻¹ until they lost their righting reflex (fig. 1). Anesthetic chamber agent concentrations were monitored continuously during the experiments using a Datex-Ohmeda Ultima Capnomac (Helsinki, Finland) and verified with gas chromatography (Model 80123B; SRI Instruments, Redondo Beach, CA). The chamber had a small door on one side, through which the animal was initially placed. The chamber also had small ports that served as the anesthetic gas inlet, the microinfusion tubing port inlet, two gas monitor sampling ports, and one gas chromatograph sampling port. Once each rat was well anesthetized, the door was partially opened, and a 25-gauge microinfusion needle was quickly inserted through the guide cannula, and the rat was placed back in the center of the chamber. The needle was attached by a polyethylene tube through the wall of the anesthetizing chamber to a 10-μl syringe (Hamilton, Reno, NV), which was driven by a minipump (Harvard Apparatus, Holliston, MA). The chamber anesthesia concentration was then lowered to 3.6% for desflurane or to 1.2% for sevoflurane. The concentration was held stable for 20 min before a microinfusion was delivered. However, if a rat showed any spontaneous movement during this stabilization period, the chamber concentration was increased by 0.1% increments until each rat remained motionless for at least 20 min. Thus, the chamber concentration varied slightly depending on a specific animal’s behavior. Rats were thus anesthetized, in separate experiments, with either desflurane (3.6 ± 0.2%; n = 55) or sevoflurane (1.2 ± 0.1%; n = 51).

Arousal Response Determinations
Responses to a single infusion of antibody per rat were graded as one of four levels: 1 = no effect-no visible movements; 2 = partial arousal - signs of arousal including eye opening and movements of extremities; 3 = full arousal-the complete turning of the animal onto its stomach, while exhibiting purposeful movements; 4 = seizures-focal or generalized tonic-clonic seizures.

Histology
Brains were sliced into 40-μm sections and stained with thionin. Microinfusions were localized blinded to behavioral data. Data were incomplete in 7 rats that expired during surgery or were euthanized due to clogged or missing cannula. Infusion sites were projected onto the -3-mm coronal brain section from the atlas of Paxinos and Watson. However, a few infusions were located within ± 1.0 mm in the anteroposterior dimension.

Statistics
The hypothesis that the CMT is involved with mediating the resumption of consciousness after antibody infusion was examined separately in both the desflurane- and the sevoflurane-exposed animals using Fisher exact test. We compared the histology of those animals showing a resumption of consciousness with those animals that...
failed to show an effect from the infusion. \( P < 0.05 \) was considered significant.

**Results**

Of the nine animals given a control antibody microinfusion, none showed any behavioral effects, regardless of cannula placement in the CMT (\( n = 4 \)) or other thalamic areas (\( n = 5 \)), data not shown. Previous control microinfusions of saline alone into the CMT were also found to be without effect.17

Overall behavioral responses to the Kv1.2 antibody are summarized in table 1. The no-effect response was seen in 30.0% of the overall proportion of rats studied; partial arousal was seen in 13.4% of the rats; full return to consciousness was seen in 16.5% of the rats. Righting occurred on average (± SD) 170 ± 99 s after the infusion and lasted a median time of 398 s (interquartile range: 279–510 s). A representative example of the resumption of consciousness is shown in figure 1 and can be seen online (see video, Supplemental Digital Content 1, which demonstrates the arousal response illustrated in fig. 1, http://links.lww.com/A823). Seizures were seen in 33% of the rats.

To assess whether the arousal reactions to the antibody represented some type of internal pain response, we also qualitatively evaluated the appearance of arousal to pain. In seven pilot animals under sevoflurane anesthesia, we tested arousal responses to a 1-mA 60-s tail-shock stimulation. The animals did move their tails and feet in response to this stimulation, and four were able to slowly curl up onto their sides during the stimulation, with two flopping onto their stomach. Yet, the qualitative nature of this type of arousal was much different from the antibody effect. It lasted only as long as the stimulus was applied, and the animals did not seem to be focally conscious, with alert looking around. With the antibody infusion, the animals appeared to regain some level of higher consciousness; they could move around in the chamber in a crawling fashion, and they responded to environmental sights and sounds. They did not appear to be in pain, as they did not seem to focus on any particular part of their body. They were somewhat uncoordinated in their movements, which might be expected; from a systems perspective, essentially nothing was done to reduce the effects of the anesthesia on their cerebellum or spinal cord areas.

![Table 1. Summary of Behavioral Responses to Intrathalamic Kv1.2 Antibody Infusion](image)

<table>
<thead>
<tr>
<th>Behavioral Response</th>
<th>Desflurane (Number of Animals)</th>
<th>Sevoflurane (Number of Animals)</th>
</tr>
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<tbody>
<tr>
<td>1, no effect</td>
<td>17</td>
<td>12</td>
</tr>
<tr>
<td>2, partial arousal</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>3, consciousness restored</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>4, seizure</td>
<td>19</td>
<td>13</td>
</tr>
</tbody>
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The histology results for desflurane are shown in figure 2, and the histology results for sevoflurane are shown in figure 3. Infusion needle-tip locations for the no-effect group versus the consciousness-restored groups for desflurane and sevoflurane are shown in figures 2A and 3A, respectively. Statistical analyses revealed that the resumption of consciousness was significantly related to infusions hitting the CMT for both agents, as also shown in figures 2B and 3B, respectively. When the infusion needle-tip was located in the CMT, 75% of those animals awoke from the anesthesia. Notably, rats having seizures often also had infusions directly into the CMT, as shown in figure 4. The animals that seized did not pass through an apparent state of progressively more arousal; rather their first movements were generally those of seizure-like activity. This was qualitatively interpreted as a dose-related effect such that too much antibody delivered directly into the CMT caused too much of a generalized excitation phenomenon for a particular rat.

The rats were allowed to recover from the infusion experiments, and all, including those that had seized, appeared to awaken normally. In subsequent days and before histologic examination, they all exhibited normal rat behaviors and were able to feed, drink, and groom normally.

**Discussion**

Microinfusing an antibody designed specifically to block the external vestibule of Kv1.2 voltage-gated po-
Potassium channels into the CMT of anesthetized rats caused a number of animals to display a temporary resumption of consciousness with restored mobility in a chamber filled with inhalational anesthesia. With histology examination of the animals' brains, it was found that an arousal response occurred in 75% of those animals where the infusion needle-tip was located within the CMT. Taken together, these findings strongly implicate the CMT as an important brain site involved with regulating levels of arousal during anesthesia and further serve to suggest that the underlying mechanism for this localized site-specific arousal effect involves anesthetic interactions with voltage-gated potassium channels.

The mechanism by which consciousness is suppressed during anesthesia remains unknown. The seminal observation by Franks and Leib in 1984 that anesthetic potency correlates with the suppression of firefly luciferase protein activity shifted the search for the molecular mechanisms of anesthesia from lipids to proteins. Many studies have since detailed how various protein ion channels are affected by numerous anesthetic substances. Yet, it remains unclear which molecular targets are the most relevant for causing the clinical effects of anesthesia. The prevailing view is that anesthetic actions on ligand-gated ion channels, such as γ-aminobutyric acid type A, glycine, neuronal nicotinic channels, N-methyl-D-aspartate, or 2-pore domain background potassium channels, are the molecular targets most directly related to producing the clinical effects of anesthetics on consciousness and immobility. Yet, a role for voltage-gated K channels in mediating the effects of anesthetics on consciousness has also been proposed.

Kv1.2 channels are densely located within the thalamus and cortex, but the early published reports suggest that they are not as densely found within the CMT as one might have anticipated from the current results. This raises the question of why exactly the CMT appeared to be the focus of the current effect. The CMT is part of the nonspecific intralaminar thalamic arousal system. It connects with brainstem areas mediating arousal and projects to wide expanses of cortical and basal ganglia areas. It receives afferents from hypothalamic areas involved with controlling sleep and arousal. Given its wide projection pattern onto cortex, it is possible that the effects found localized to the CMT area represent an influence on the projections to or from this area (or fibers of passage), rather than on the CMT cell bodies themselves. The large number of seizures found with injections around the midline thalamic area support the idea that this region is involved in regulating overall levels of cortical excitability. The findings reported here suggest that voltage-gated potassium channels in the CMT may contribute more to regulating arousal through localized network interactions than previously thought. Small effects on these channels can have large system-wide effects within neural networks and brain systems for which spike timing is a critical element of the transmission of information.

However, it is important to clarify a number of issues related to these findings. First, the findings are primarily significant for adding further support to the idea that the CMT is an important node in an arousal network that may directly or indirectly interact with anesthesia. The amount of infusion volume used was only 0.5 μl. This is a much smaller volume than many in vivo studies use, and it suggests that the effects found are localized to a
very small area immediately around the infusion sites that encompasses a size of less than approximately 0.25–0.5 mm. This small infusion volume was used to minimize spread from the needle-tip and help provide localization of the effects. A number of the infusions that hit the CMT or were near it did not cause an arousal reaction. This is likely due to the delivery of an insufficient dose with a particular infusion. The CMT interacts with both ascending (from brainstem),46,47 and descending (from cortex) arousal pathways.48 From an anesthesia perspective, the CMT receives input from the hypothalamus,40 a connection that helps modulate the sedation effects of anesthetics through GABAergic or orexinergic effects.18,49 It also interacts with the mesopontine tegmental anesthesia area,47 a brainstem region that induces an apparent anesthetic-like state when microinfused with barbiturate.50 Pharmacologic manipulations of the CMT can both directly enhance arousal and produce sedation effects.42,51 Microinfusion of nicotine into the CMT of anesthetized rats restores behavioral arousal despite continued anesthetic exposure.17 Microinfusion of a γ-aminobutyric acid agonist muscimol is reported to cause a sedation response.52 Taking these facts together with the current findings strongly suggests that the CMT is intimately involved with regulating levels of arousal during anesthesia.

Second, it should not be assumed without much further work that the arousal effect associated with the infusion of the Kv1.2 channel blocking antibody into the CMT is directly related to antagonism of a specific mechanism of anesthesia. This is certainly one possibility, but other evidence suggests this is an unlikely possibility. In vitro studies examining the effects of anesthetics on voltage-gated potassium channels show that these channels are affected by anesthetics,7 but generally only at doses much greater than those that are clinically relevant.53 One exception to this generalization is evident for Shao2 mutant Kv channels, which appear to be highly sensitive to certain anesthetics.8 Yet, the effects on these and most other Kv channels is generally one of current blockade. Thus, in the present work, if anesthesia is acting to block currents through the Kv channels and the Kv1.2 antibody is also acting to block currents, then it seems more likely that the antibody should have enhanced sedation, rather than causing an arousal reaction. Nevertheless, the answer to this apparent contradiction may lie in the extreme biologic diversity of Kv channels, where it could be speculated that some heteromeric subunit combinations may exist that can produce anesthetic-sensitive channels that do open in response to anesthetic exposure. Another speculation further illustrates the potentially indirect nature of these findings. Assuming that the antibody did block the Kv1.2 channels in the CMT and functionally eliminated them, this would act to acutely change the firing patterns of the CMT neurons involved, but it would not stop the actions of the same anesthetic from affecting any other anesthetic-sensitive channels, such as GABAergic, glycineergic, cholinergic or K2P channels. The ultimate effect on thalamic neuronal firing patterns is likely the result of the combined contributions of multiple influences on the cell membrane potential.7,54 therefore, the blocking of the Kv1.2 channel can be seen as just one additional influence that changes the cell’s membrane potential and hence its likelihood of entering a particular pattern of action potential firing.

Third, generalized nonspecific effects of an antibody infusion into the CMT are unlikely to be the source of the arousal responses, as the control antibody infusions did not cause any reactions. In addition, a nonspecific arousal effect due to some type of internal pain-like state is unlikely because the qualitative nature of an arousal response to a painful stimulation was much different from that seen with the antibody effect. However, nonspecific binding effects of the Kv1.2 antibody cannot be ruled out. Antibodies are affinity reagents. This means that even though they have tremendous affinity for their target antigen, some level of low-affinity crossreactivity to other closely related protein sequences is common and often contributes to the signal. Thus, it is likely that most of the Kv1.2 antibody bound to the Kv1.2 receptor, but it might also have bound to other similar receptors or even to other similar channels. It is often seen with immunohistochemistry that nonspecific binding can occur to an extent that is sufficient to overwhelm the intended signal of interest.

How specific is this polyclonal antibody for blocking only Kv1.2 ion channels? From the original Zhou et al. work, the specificity between Kv1.2 and Kv1.3 is quite good.25 Yet, the antibody was not tested directly against Kv1.1 or Kv1.6 channels. This may be important because Kv1.1, Kv1.2, and Kv1.6 channels all show similar affinity for the binding of α-dendrotoxin.54 On this basis alone, it would be reasonable to assume that some crossreactivity of the Kv1.2 channel blocking antibody with the Kv1.1 and Kv1.6 channels may have occurred. Only further work with more specific versions of various toxins44 or the development of monoclonal antibodies might help clarify to what extent some crossreactivity may have influenced these findings.30,55 Indeed, the development of monoclonal antibodies for use in the specific blocking of various channels in vivo is now almost routine,28,31,56 though the polyclonal approach remains an established technique.29

If one speculates that the unconsciousness of anesthesia occurs through the opening of thalamic Kv1.2 channels and that the antibody blocked this open pore to restore consciousness, then why did the suppression of the unconsciousness response not last indefinitely? It is conceivable that the antibody may have dissociated from the receptor in a relatively short period of time, but this seems unlikely given the nature of antibody binding.
More likely would be the possibility that receptor trafficking played some role in the termination of the response.\(^5^7\) In addition, the arousing response itself may have been due to presynaptic actions serving to decrease the functioning of GABAergic neurons, causing a temporary inhibition of inhibition or a disinhibition reaction.\(^5^8,^5^9\) Another possibility is that a nonspecific generalized arousal reaction centered on the midline thalamus may have contributed to the response, such has been seen when acute ibotenic acid infusions are given into the midline thalamus.\(^6^0\) Further testing with other excitatory substances such as glutamate and even potassium itself would seem warranted.

One approach to identify appropriate targets of anesthetic action is to genetically modify specific ion channels and then evaluate the behavioral effects of such mutations on the various end-points of anesthesia in the mutant animals.\(^6^1\) Another relatively new approach is to use antibody-based validation of relevant ion channel drug targets.\(^3^0\) This approach has a number of potential advantages for use in anesthesia research. (1) After antibody development, the target selectivity and specificity of binding is essentially unparalleled. (2) Normal wild-type animals can be studied. This eliminates the fear that some compensatory mechanisms might interact with the behavior of interest, as may be the case with mutant animals. (3) The behavioral effects of any particular antibody-binding response can be determined, and then the underlying regional and even neuronal site-specificity associated with the responses can be identified with immunohistochemical techniques. The antibody approach, however, is not without its limitations. Proper validation and development of target specificity is enormously expensive, time consuming, and generally beyond the means and experience of most investigators.\(^3^0\)

Thus, antibodies are usually borrowed from colleagues who have already developed them (i.e., limited availability), or they are commissioned from an antibody reagent company. The quality control on such products can vary substantially, and the inadvertent use of a not-so-specific antibody can prove greatly misleading.\(^6^2\) Nevertheless, the antibody approach may represent one pathway out of the quagmire that currently complicates the mechanism(s) of anesthesia research field.

Finally, it is important to note that this is not the first demonstration to link a site-specific change in potassium channel functioning to the hypnotic component of anesthesia and show an ability to antagonize the consciousness-suppressing effect of an anesthetic. Infusions of various K channel blockers, dendrotoxin (KV), charybdotoxin (KCa), and quinine (KCa and Kv) were found to reduce the hypnotic effect of the selective \(\alpha_2\)adrenergic agonist dexmedetomidine when they were delivered discretely into the locus coeruleus of rats.\(^6^3\) Taken together with the current findings, the ability of interactions with voltage-gated potassium channels to antagonize the hypnotic component of at least three different anesthetic agents (i.e., sevoflurane, desflurane, and dexmedetomidine) \textit{in vivo} would seem to identify these channels as prime targets in need of further study.

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