General anesthetics activate a nociceptive ion channel to enhance pain and inflammation

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General anesthetics (GAs) have transformed surgery through their actions to depress the central nervous system and blunt the perception of surgical insults. Counterintuitively, many of these agents activate peripheral nociceptive neurons. However, the underlying mechanisms and significance of these effects have not been explored. Here, we show that clinical concentrations of noxious i.v. and inhalation GAs excite sensory neurons by selectively activating TRPA1, a key ion channel in the pain pathway. Further, these GAs induce pain-related responses in mice that are abolished in TRPA1-null animals. Significantly, TRPA1-dependent neurogenic inflammation is greater in mice anesthetized with pungent compared with nonpungent anesthetics. Thus, our results show that TRPA1 is essential for sensing noxious GAs. The pronociceptive effects of GAs combined with surgical tissue damage could lead to a paradoxical increase in postoperative pain and inflammation.

TRPA1 | TRPV1 | isothiocyanate | mustard oil | isoflurane

General anesthetics (GAs) are a diverse group of chemicals with the shared ability to suppress CNS activity and induce reversible unconsciousness (1). This immensely useful pharmacological property permits the >100 million surgeries performed worldwide each year. The molecular mechanisms of anesthesia have been extensively studied and there is now considerable evidence that GAs can inhibit CNS activity by discrete actions on membrane ion channels, in particular, through the activation of γ-aminobutyric acid (GABA) receptors (1). Strikingly, and in contrast to their inhibitory effects in the CNS, some GAs can stimulate peripheral nociceptors. For example, the i.v. anesthetics propofol and etomidate elicit “burning” pain on injection (2–4). Further, inhalation or volatile GAs (VGAs) can excite Aδ- and C-fiber neurons innervating the rabbit cornea (5), monkey skin (6), and canine airways (7). Indeed, neurogenic respiratory irritation limits the use of the more pungent anesthetics as induction agents (8, 9). These excitatory effects of GAs on sensory nerves may explain, in part, why subanesthetic concentrations of these agents are hyperalgesic in rodents (10) and in humans (11). Of particular clinical relevance, the administration of GAs coincides with surgically induced tissue damage, and the combination of nociceptor activation/sensitization and tissue injury has important implications for postsurgical pain and inflammation. Despite the potential importance of these effects, the underlying mechanisms and consequences of anesthetics activating nociceptors are yet to be determined.

Interestingly, VGAs appear to selectively activate the capsaicin-sensitive population of sensory neurons (7), suggesting that a receptor localized to these cells transduces the noxious effects of these agents. Several members of the Transient Receptor Potential (TRP) ion channel family are attractive candidates. TRPV1, expressed in ∼50% of nociceptive sensory neurons, responds to chemical irritants including capsaicin, protons, salt, and ethanol (12). TRPA1, coexpressed with TRPV1 in 25–30% of nociceptors (13), is a specific neuronal target for mustard oil (allyl isothiocyanate, AITC), wasabi (14), and a number of volatile irritants, including garlic (15, 16) and acrolein, a toxic component of tear gas (13, 14). In addition to these exogenous agents, endogenous inflammatory mediators also engage these channels. Indeed, both receptors contribute to neurogenic inflammation and pain signaling; disruption of the TRPV1 gene abolishes thermal hyperalgesia (17, 18) whereas deletion of TRPA1 impairs bradykinin-induced nociception (13, 19).

In this study we tested the hypothesis that these nociceptive TRPs are important sensory nerve targets for pungent GAs. Further, we explored whether this form of signaling underlies anesthetic-evoked nociception and contributes to nerve-mediated inflammation during anesthesia.

Results

Noxious Volatile and Intravenous GAs Activate TRPA1. Several VGAs are known to stimulate nociceptors and we asked whether this is mediated by TRP channels. Application of the pungent agent isoflurane [0.9 mM, or 2.9 minimum alveolar concentration (MAC)] produced inward currents in voltage-clamped, TRPA1-expressing HEK293 cells, but failed to activate TRPM8 and TRPV1 (Fig. 1A). Similarly, isoflurane (0.9 mM, 2.9 MAC) evoked currents in 11 of 35 (31%) neurons from wild-type mice tested under voltage-clamp, and 10 (91%) of these cells were also sensitive to AITC (Fig. 1B). Isoflurane activated TRPA1 in a dose-dependent manner (Fig. 1C), with an EC50 of 0.18 ± 0.02 mM (0.57 MAC). Thus, these effects of isoflurane occur at relevant clinical concentrations (∼1–3 MAC).

Next, we compared VGAs possessing differing pungencies for activity at TRPA1. Interestingly we found that the pungent anesthetics, isoflurane and desflurane, robustly activated the channel, whereas the nonpungent agents sevoflurane and halothane were without effect (Fig. 1E). This relationship replicates the perceived pungency of VGAs when administered to patients (8). These effects of isoflurane and desflurane were retained in cell-free patches from TRPA1-expressing HEK293 cells and AITC-sensitive neurons (Fig. 1D); both VGAs enhanced single-channel gating, but also reduced the single-channel conductance from ∼110 pS to ∼60 pS (0.23 mM isoflurane) and ∼80 pS (0.9 mM desflurane). This block was voltage-dependent and relieved at depolarized potentials (supporting information (SI) Fig. S1). Thus, these agents (isoflurane, in particular) produce both agonistic and pore-blocking actions at TRPA1, and this explains the bimodal dose–response relationship that shows a peak at ∼1 mM and a reduction at higher concentrations of isoflurane (Fig. 1C).

The i.v. GAs, propofol and etomidate, are associated with...
marked pain on injection (2–4). This pain occurs in 80% to 90% of patients; however, the underlying mechanisms are unknown. We asked whether propofol and etomidate could excite sensory neurons through a direct modulation of TRP channels. In voltage-clamped HEK293 cells (membrane potential, −50 mV) both propofol and etomidate (100 μM) produced a robust activation of TRPA1 but were without effect on TRPV1 or TRPM8 channels (Figs. 2A and B). This activation occurred over the clinically relevant concentration range of 1–100 μM (Fig. 2D); the free concentration of propofol in clinical formulations is ≈100 μM (4). Interestingly, on washout of propofol there was a surge in current suggesting an additional pore-blocking effect of the anesthetic (Fig. 2A). Accordingly, single-channel measurements showed that propofol both increased TRPA1 activity and reduced the unitary conductance (Fig. 2E). As with the inhalation agents this block was voltage-dependent (Fig. S1B). Thus, responses to propofol were greater at depolarized potentials, ~8% and 38% of the full agonist, AITC, at −150 mV and +200 mV, respectively (Fig. 2C). We also observed propofol-evoked inward currents in AITC-sensitive DRG neurons (n = 6; Fig. 2F) and these currents were sensitive to a TRPA1 inhibitor, camphor. Furthermore, propofol depolarized these neurons under current clamp to elicit action potentials (Fig. 2G). To explore whether propofol could sensitize TRPV1 and TRPM8, we examined its effect on voltage-dependent activation. Propofol and etomidate (100 μM) produced a small reduction in the half-maximal voltage ($V_{1/2}$) for TRPV1 activation of 10.5 and 9.3 mV, respectively (n = 4–5). Propofol was without effect on TRPM8 ($\Delta V_{1/2} = 1.7$ mV, n = 6), whereas etomidate increased the $V_{1/2}$ by 25.5 mV (n = 5). Thus, the predominant action of these GAs is to activate TRPA1, but etomidate can additionally block TRPM8.

Fig. 1. Volatile GAs activate TRPA1. (A) Representative current traces during application of isoflurane (0.9 mM, 2.9 MAC) in HEK293 cells expressing TRPM8, TRPV1, or TRPA1. Positive responses were elicited by menthol (1 mM), capsaicin (1 μM), or AITC (100 μM). (B) Isoflurane (0.9 mM) evoked inward currents in AITC-sensitive sensory neurons (n = 11). (C) Isoflurane activates TRPA1 in a dose-dependent manner with an EC50 of 180 ± 20 μM (n = 4–7) and a Hill coefficient of 1.6 ± 0.2. At 2.7 mM isoflurane the response is reduced reflecting an additional blocking mechanism. (Inset) Example of washout of isoflurane; scale bars: 100 pA and 5 s. (D) Isoflurane (0.25 mM) and desflurane (0.9 mM) activate single TRPA1 channels in outside-out patches from HEK293 cells (no activity was observed in mock-transfected cells). The $V_{m}$ was +50 mV. All-points histogram from 2-s data segments are shown on the right. (E) The mean currents (fraction of isoflurane) evoked by 0.9 mM concentrations of halothane, sevoflurane, and desflurane. Data are mean from five to six experiments.

**GAs Excite Sensory Nerves by Selectively Activating TRPA1.** Next, to determine whether TRPA1 is the primary sensory nerve target for irritant GAs we performed calcium imaging in DRG neurons. Fig. 3A shows that desflurane (1.5 mM, 3 MAC) evoked a Ca2+ increase in a subset of neurons cultured from wild-type mice (36 of 123 cells). These desflurane-responsive cells were all sensitive to AITC. In contrast, no responses to desflurane were evident in cells obtained from TRPA1-null mice (Fig. 3B; n = 125). Thus, TRPA1 appears to be essential for transducing the excitatory effect of VGAs in sensory neurons. We performed similar calcium-imaging analysis with propofol. Fig. 3C shows that propofol selectively evoked a Ca2+ rise in AITC-sensitive neurons, with ~30% of cells exhibiting dual sensitivity to propofol and AITC. In contrast, no responses to propofol were observed in neurons cultured from TRPA1-null mice (Fig. 3D; n = 120). Furthermore, a total of 43% of these TRPA1-deficient cells were sensitive to capsaicin (Fig. 3B and D), thereby excluding a significant contribution of TRPV1 in desflurane and propofol signaling. Taken together, the data indicate that TRPA1 is a major determinant of the sensory nerve excitation produced by noxious GAs.

**VGAs Directly Activate TRPA1.** GAs could potentially modulate TRPA1 by modulating [Ca2+]i or cellular signaling cascades. The presence of extracellular Ca2+ enhanced the response to GAs (Fig. S2); however, activation persisted when Ca2+ was removed (and with 5 mM intracellular EGTA), indicating a Ca2+-independent mechanism. Further, we found that both volatile and i.v. GAs effectively modulated TRPA1 in cell-free patches (Figs. 1D and 2E) suggesting that these anesthetics signal in a membrane-delimited fashion, not via a soluble second messenger. Indeed, there is accumulating evidence that GAs can directly regulate ligand-gated ion channels. VGAs and alcohols...
share a common binding pocket in GABA\(_A\) and glycine receptors, located between transmembrane domains 2 and 3 (20, 21). Interestingly, alcohol modulation of these receptors exhibits a carbon chain-length “cutoff”; the potency of alcohols increase with carbon chain length up until this cutoff, after which further increases in molecular size no longer increase alcohol potency (20, 21). These data are consistent with the existence of a cavity on these proteins that is accessible only to alcohols of a finite molecular volume. We observed a similar cutoff with TRPA1. Fig. 4 A and B shows that alcohols of 6–12 carbons enhanced activation of TRPA1 with a cutoff between octanol and decanol. Next, we explored whether alcohols and VGAs act at similar binding site(s) on TRPA1. We predicted that these chemicals would produce an additive response if they acted at different

![Image](image1)

**Fig. 2.** Noxious i.v. GAs activate TRPA1. (A and B) In HEK293 cells, propofol and etomidate (100 \(\mu M\)) selectively activate TRPA1 without affecting TRPM8 or TRPV1 currents \((V_m = -50 \text{ mV}, n = 6-8)\). (C) \(I-V\) relationship for responses to propofol and AITC (1 mM, \(n = 7\)). (D) Dose-dependent activation by propofol (0.3–300 \(\mu M\), \(n = 4–6\)). (E) Propofol (100 \(\mu M\)) activates single TRPA1 channels in an outside-out patch \((n = 3, V_m = +40 \text{ mV})\). All-points histograms reveal a decrease in unitary conductance from 108 to 94 pS. (F and G) Propofol (100 \(\mu M\)) evoked inward currents and depolarized AITC-sensitive DRG neurons \((n = 6)\). Currents were blocked by camphor (0.5 mM).

![Image](image2)

**Fig. 3.** GAs excite DRG neurons via TRPA1 (A and C) (Left) Representative Ca\(^{2+}\) transients evoked by desflurane (1.5 mM, 3 MAC), propofol (100 \(\mu M\)), and AITC (1 mM) in DRG neurons obtained from wild-type mice. (Right) The percentage of neurons responsive to desflurane \((n = 123)\), propofol \((n = 63)\), and AITC. (B and D) (Left) Representative Ca\(^{2+}\) transients evoked by desflurane propofol and capsaicin (100 nM) in DRG neurons obtained from TRPA1-null mice. (Right) The number of DRG neurons responsive to desflurane \((n = 125)\), propofol \((n = 120)\), or capsaicin.
In contrast to AITC, we found that successive applications of their chemical structures do not support such a mechanism. More-
AITC vapors in the chamber. These effects of isoflurane and sevoflurane on AITC-evoked inflammation paralleled the effect of these VGAs on AITC-evoked currents. Fig. 6C shows that the pungent agents isoflurane and desflurane markedly enhanced AITC-evoked currents in TRPA1-expressing oocytes, whereas sevoflurane and another nonpungent VGA, methoxyflurane, did not. Thus, the level of AITC-evoked inflammation during anesthesia correlates with the ability of VGAs to potentiate TRPA1.

Taken together, these data suggest that VGAs, when administered in vivo, can differentially modulate TRPA1 to modulate neurogenic signaling.

Discussion
In this study we reveal the mechanism by which GAs excite sensory neurons. Our data indicate that clinical doses of noxious GAs respectively activate and sensitize the mustard-oil receptor TRPA1, a principal receptor in the pain pathway. Although several exogenous, pungent compounds are known to activate these channels, including garlic (15, 16), wasabi (14), and formalin (27), GAs arguably represent a more medically significant class of agonists because they are administered systemically to patients at a time of extensive tissue injury.

GAs appear to regulate TRPA1 directly and several lines of evidence support this idea. First, we found that GAs remained effective in cell-free patches suggesting that they signal in a membrane-delimited fashion and not via a soluble second messenger. Second, VGAs modulate TRPA1 in proportion to their pungency (desflurane ≳ isoflurane ≳ sevoflurane/halothane) and this does not correspond with their ability to partition into the membrane (halothane/sevoflurane ≳ desflurane). This argues against VGAs signaling via membrane fluidity. Third, we observed that long-chain alcohols, like VGAs, produced a similar modulation of TRP channels and exhibited a cutoff between 8 and 10 carbons. The existence of discrete cutoff values is consistent with binding of alcohols to a volume-restricted cavity on TRPA1 protein. Further, our finding that alcohols and VGAs exhibit a similar activation profile at different TRP channels, and activate TRPA1 in a nonadditive manner, supports the idea that these compounds act through the same mechanism. Propofol, in contrast, produced an additive response and appears to act via a distinct site(s).

Our data indicate that TRPA1 is responsible for the acute noxious effects of GAs. Several VGAs produce airway irritation when administered to patients. Desflurane and isoflurane irritate when inhaled at >1 MAC and 1.5 MAC, respectively, whereas sevoflurane and halothane do not provoke irritation at any concentration (8, 9). Consequently, sevoflurane is preferred as an inhalation induction agent and desflurane is avoided. In addition, the i.v. GAs propofol and etomidate are associated with marked pain on injection, especially if a vein on the dorsum of the hand is used, and this causes considerable distress to patients (2–4). We show that these noxious inhalation and i.v. GAs activate and sensitize TRPA1, whereas nonpungent VGAs are without effect. Moreover, these agents excite sensory neurons in a TRPA1-dependent manner; the excitatory effect is absent in TRPV1-deficient neurons. Furthermore, we show that propofol-induced pain behaviors are abolished in TRPA1-null animals. Thus, TRPA1 appears essential for GA-evoked nociception. On a practical note, these data suggest that selective TRPA1 antagonists may represent an effective treatment strategy for preventing the pronociceptive effects of GAs. Currently, lidocaine pretreatment is the most popular method for reducing propofol/etomidate pain, but cannot entirely eliminate the problem.

Significantly, our data suggest that, in addition to these acute noxious effects, VGAs may also sensitize nociceptors during anesthesia maintenance. Surgical insults produce TRPA1 activators such as bradykinin (13, 28) and aldehydes (29, 30), which combined with VGAs could lead to an increased release of neuropeptides from peripheral terminals, culminating in greater neurogenic inflammation. Consistent with this hypothesis, our data show that AITC-evoked neurogenic inflammation is greater in animals anesthetized with the pungent GA, isoflurane, than with the nonpungent agent, sevoflurane. In addition, elevated sensory nerve activity can drive central sensitization—a form of pain plasticity (31)—ultimately producing a medium- to long-lasting facilitation of nociceptive processing in the spinal cord. Thus, through these TRPA1-dependent mechanisms, pungent VGAs could produce greater postsurgical pain and inflammation than their nonpungent counterparts.

In summary, our results show that GAs excite sensory neurons by selectively activating TRPA1. These findings explain the acute noxious properties of i.v. and inhalation GAs and reveal a pronociceptive effect of GAs that may occur during surgery.

Materials and Methods
Electrophysiology. HEK 293F cells were transfected with rat TRPV1, TRPA1, and TRPM8 (gift of David Julius, University of California, San Francisco). Dorsal root
ganglia were cultured from adult mice (C57Bl/6J wild type and TRPV1-null, and mixed B6/129 background TRPA1-null) in Neurobasal + 2% B-27 medium (Invitrogen). 0.1% L-glutamine and 1% penicillin/streptomycin. Whole-cell and single-channel patch-clamp recordings were performed by using an EPC8 amplifier (HEKA Electronics). For whole-cell and excised patch recordings the bath solution contained 140 mM NaCl, 4 mM KCl, 1 mM MgCl2, 1.2 mM CaCl2, 10 mM Hepes, 10 mM glucose, pH 7.3. For neuronal recording NaCl was replaced with K-glucoside (plus 1 mM ATP, 0.2 mM GTP). Solutions were applied via a gravity-fed system. Separate outlets were used to apply capsaicin and AITC solutions to avoid contamination. Voltage-dependent properties were measured as described in ref. 32. Current–voltage measurements comprised a 200-ms ramp from −150 to 200 mV. The baseline currents under control conditions were subtracted. For cell-attached experiments, peak amplitudes were measured from all-points histograms, and open probability was measured as NP, > 750 ms. Defolliculated Xenopus laevis oocytes were injected with −10 ng of hTRPA1 (gift of Ardath Patapouvian, The Scripps Research Institute, La Jolla, CA). Oocytes were placed in a Perspex chamber and continuously superfused (5 ml/min) with Ca2+-free solution containing 100 mM NaCl, 2.5 mM KCl, 5 mM Hepes, 1 mM MgCl2 and titrated to pH 7.3 with −5 mM NaOH.

Ca2+ Imaging. Neurons were loaded with 1 μM Fluo4-AM (Molecular Probes) for 20 min and washed for a further 10–20 min before recording. The dye was excited at 488 ± 15 nm. Emitted fluorescence was filtered with a 535 ± 25 nm bandpass filter, captured by a SPOT RT digital camera (Diagnostic Instruments) and read into a computer. Analysis was performed offline by using Simple PCI software (Compuix Inc.).

Behavioral Experiments and Neuroinflammation. Animal experiments were performed according to National Institutes of Health and institutional guidelines. Propofol (50% in mineral oil, 20 μl) was applied to the nasal epithelium of male C57/Bl6 and TRPV1-null mice and mixed B6/129 background TRPA1-null (and mixed B6/129 back-ground TRPA1-/- mice (5–7 weeks). Nociceptive behavior (nose wiping in sawdust bedding, see Movie S1) was recorded for 2 min with a video camera and the duration was subsequently measured by a blinded observer. Application of capsaicin (10 mM) produced similar behavior in wild-type but not in TRPV1-null mice, establishing that this is a bona fide nocifensive behavior. Electromyographic (EMG) activity was recorded via platinum electrodes from the semitendinous muscle in mice anesthetized with urethane (1.3 g/kg) as described in ref. 25. The EMG signal was recorded by using a low-pass cutoff frequency of 200 Hz and integrated offline by using a 100-ms time window. To induce the flexor reflex response, 30 μl of vehicle (0.01% DMSO), propofol (500 μM), or capsaicin (50 μM) were administered at a 5-min interval into the femoral artery via a PE10 catheter. Neurogenic inflammation was induced in male C57Bl6 mice (6–8 weeks) with 20 μl of mustard oil (0.6%) applied to the front and back surface of one ear, and mineral oil was applied to the other (26). Animals were anesthetized with isoflurane or sevoflurane in oxygen by using anesthetic-specific vaporizers (Vapomatic); the concentrations in the chamber were maintained at −1.2 MAC confirmed with a gas analyzer (Ohmeda). Ear thickness was recorded by using an engineer’s micrometer (Mitutoyo Corp.) before mustard-oil application and thereafter every 15 min for 60 min of anesthesia and 60 min of recovery.

Volatile General Anesthetics and Chemicals. Saturated stock solutions of volatile GAs were prepared in gas-tight bottles by dissolving excess anesthetic agents in bath solutions over-night. These stock solutions fresh dilutions were made up every 40–60 min. Concentrations of GAs in the bath solutions were verified by using a modified head-space gas chromatography method. The equivalent MACs were calculated by using published conversion factors reported for halothane (0.27 mM), isoflurane (0.31 mM), desflurane (0.51 mM), sevoflurane (0.35 mM), and enfurane (0.64 mM) in rat at 37°C (33). Alcohols with <6 carbons were dissolved directly into extracellular solution, and alcohols containing 6 carbons or more were dissolved in DMSO and then diluted into extracellular solutions that were sonicated for 20 min. All other drugs were prepared as stock solutions in DMSO or ethanol and diluted into physiological solution before experiments. Drug vehicles in final recording solutions were 0.05–0.1% DMSO or ethanol, concentrations with no tested biological effect at TRP channels used in this study.

Statistical Analysis. Data are given as mean ± SEM. and statistical significance was evaluated by using ANOVA or Student’s t test.

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