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

José A. Matta*, Paul M. Cornett*, Rosa L. Miyares*, Ken Abe†, Niaz Sahibzada*, and Gerard P. Ahern*‡

Departments of *Pharmacology and †Physiology and Biophysics, Georgetown University, 3900 Reservoir Road, NW, Washington, DC 20007

Edited by Ramón Latorre, Centro de Neurociencias, Universidad de Valparaíso, Valparaíso, and approved April 21, 2008 (received for review November 21, 2007)

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. 1*A*). 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. 1*B*). Isoflurane activated TRPA1 in a dose-dependent manner (Fig. 1*C*), with an EC_{50} 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. 1*E*). 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. 1*D*); 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. 1*C*).

The i.v. GAs, propofol and etomidate, are associated with

Author contributions: J.A.M., P.M.C., and G.P.A. designed research; J.A.M., P.M.C., R.L.M., K.A., N.S., and G.P.A. performed research; J.A.M., P.M.C., R.L.M., N.S., and G.P.A. analyzed data; and J.A.M., R.L.M., and G.P.A. wrote the paper.

Conflict of interest: Georgetown University has filed a provisional patent relating to this study.

This article is a PNAS Direct Submission.

‡To whom correspondence should be addressed. E-mail: gpa3@georgetown.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0711038105/DCSupplemental.

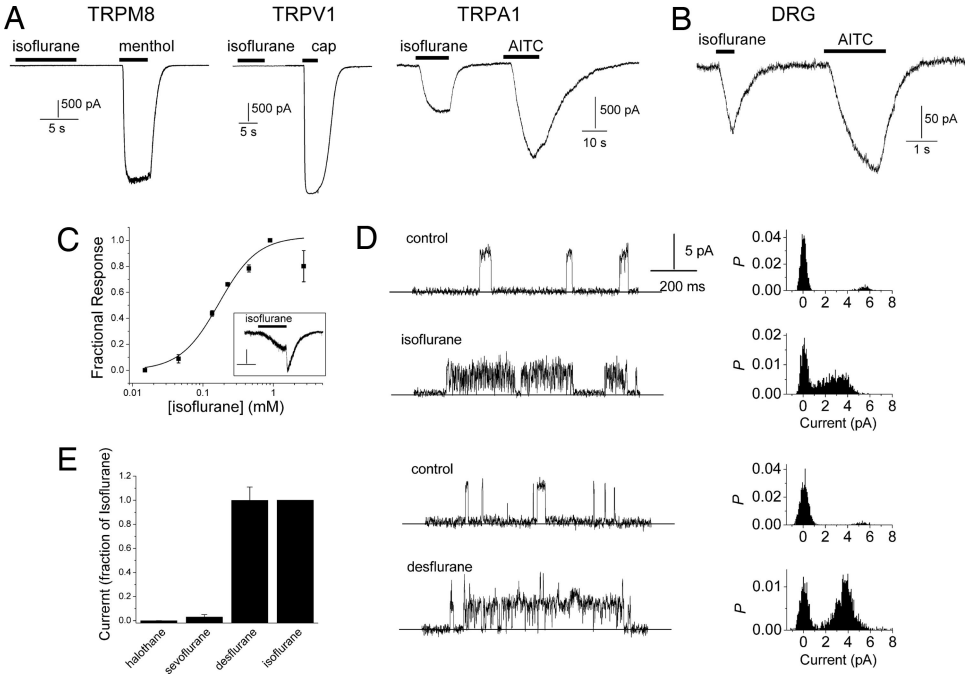

Fig. 1. Volatile GAs activate TRPA1. (*A*) Representative current traces during application of isoflurane (0.9 mM, 2.9 MAC) in HEK293 cells expressing TRPM8, TRPV1, or TRPA1. Positive responses were elicited by menthol (1 mM), capsaicin (1 μM), or AITC (100 μM). (*B*) Isoflurane (0.9 mM) evoked inward currents in AITC-sensitive sensory neurons ($n = 11$). (*C*) Isoflurane activates TRPA1 in a dose-dependent manner with an EC_{50} of 180 $\pm$ 20 μM ($n = 4$–7) and a Hill coefficient of 1.6 $\pm$ 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.

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. 2*A* and *B*). This activation occurred over the clinically relevant concentration range of 1–100 μM (Fig. 2*D*); 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. 2*A*). Accordingly, single-channel measurements showed that propofol both increased TRPA1 activity and reduced the unitary conductance (Fig. 2*E*). As with the inhalation agents this block was voltage-dependent (Fig. S1*B*). 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. 2*C*). We also observed propofol-evoked inward currents in AITC-sensitive DRG neurons ($n = 6$; Fig. 2*F*) and these currents were sensitive to a TRPA1 inhibitor, camphor. Furthermore, propofol depolarized these neurons under current clamp to elicit action potentials (Fig. 2*G*). 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.

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. 3*A* shows that desflurane (1.5 mM, 3 MAC) evoked a Ca^{2+} 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. 3*B*; $n = 125$). 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. 3*C* shows that propofol selectively evoked a Ca^{2+} 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. 3*D*; $n = 120$). Furthermore, a total of 43% of these TRPA1-deficient cells were sensitive to capsaicin (Fig. 3 *B* 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 $[Ca^{2+}]_i$ or cellular signaling cascades. The presence of extracellular Ca^{2+} enhanced the response to GAs (Fig. S2); however, activation persisted when Ca^{2+} was removed (and with 5 mM intracellular EGTA), indicating a Ca^{2+}-independent mechanism. Further, we found that both volatile and i.v. GAs effectively modulated TRPA1 in cell-free patches (Figs. 1*D* and 2*E*) 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

PHARMACOLOGY

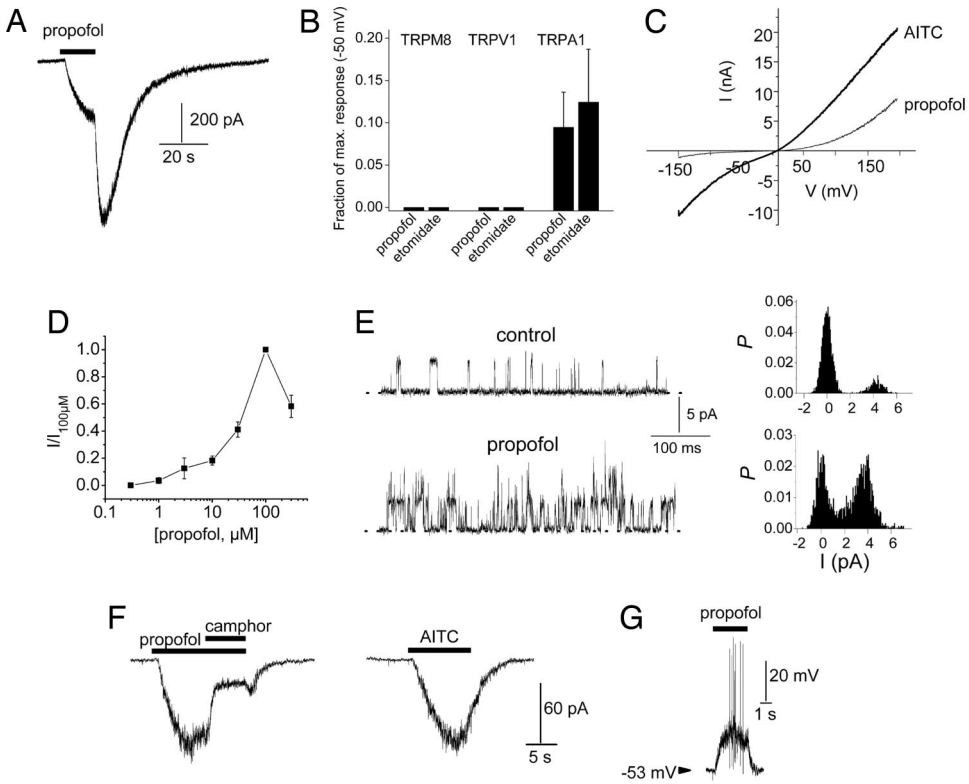

Fig. 2. Noxious i.v. GAs activate TRPA1. (*A* and *B*) In HEK293 cells, propofol and etomidate (100 μM) selectively activate TRPA1 without affecting TRPM8 or TRPV1 currents ($V_m = -50$ mV, $n = 6–8$). (*C*) *I–V* relationship for responses to propofol and AITC (1 mM, $n = 7$). (*D*) Dose-dependent activation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

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

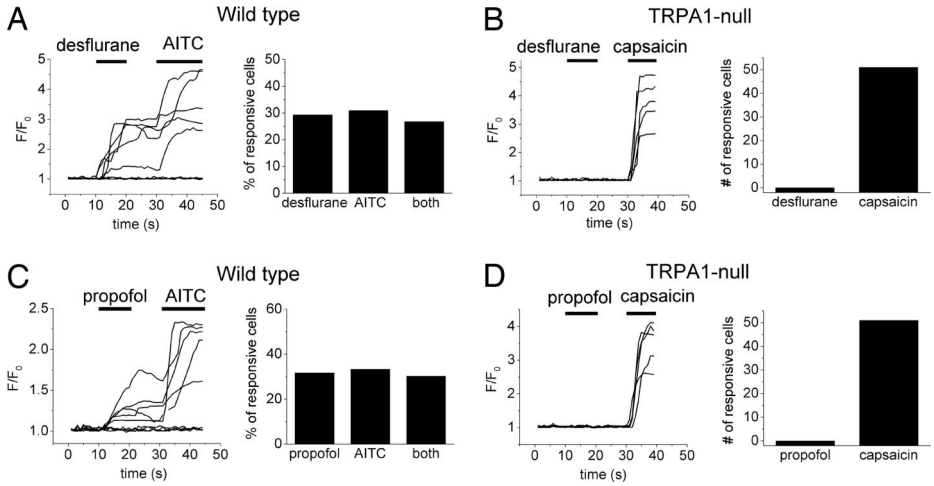

Fig. 3. GAs excite DRG neurons via TRPA1 (*A* and *C*) (*Left*) Representative Ca^{2+} transients evoked by desflurane (1.5 mM, 3 MAC), propofol (100 μm), and AITC (1 mM) in DRG neurons obtained from wild-type mice. (*Right*) The percentage of neurons responsive to desflurane ($n = 123$), propofol ($n = 63$), 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.

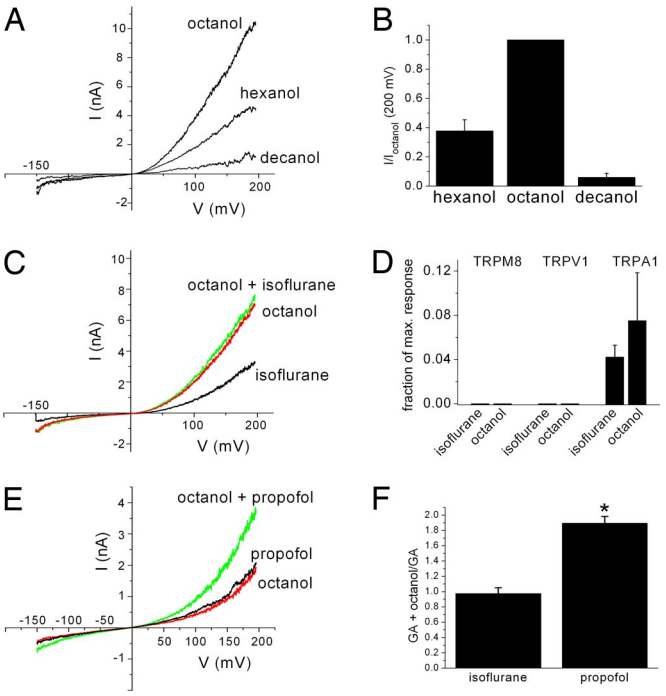

Fig. 4. Volatile anesthetics interact directly with TRPA1 channels. (*A* and *B*) Activation of TRPA1 by hexanol (3 mM), octanol (1 mM), and decanol (0.6 mM) ($n = 4$). (*C*) Octanol (1.8 mM) and isoflurane (0.9 mM) modulate TRPA1 in a nonadditive fashion. (*D*) Activation of TRPM8, TRPV1, and TRPA1 currents at −50 mV by isoflurane (0.9 mM) and octanol (1 mM), compared with maximal stimulation with menthol (1 mM), capsaicin (1 μM), and AITC (1 mM), respectively ($n = 4–6$). (*E*) Propofol (100 μM) and octanol (1.8 mM) produce an additive response at TRPA1. (*F*) Mean effects of octanol (1.8 mM) on isoflurane (0.9 mM) and propofol (100 μM)-evoked currents ($n = 5–6$), *, $P < 0.01$.

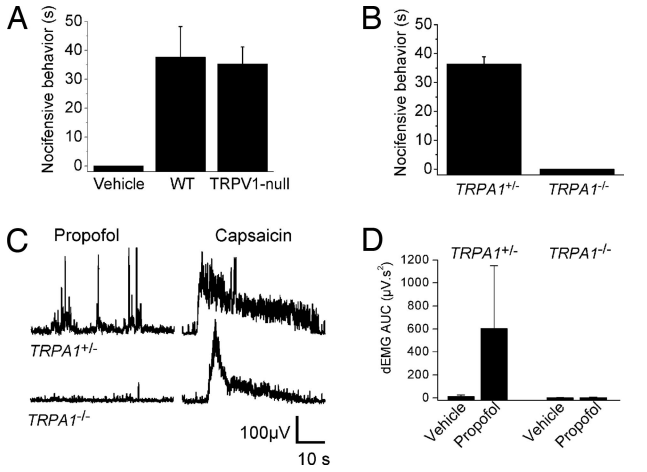

Fig. 5. TRPA1 mediates propofol-evoked, pain-related behavior. (*A*) Topical application of propofol (50%) to the nasal epithelium evokes nocifensive behavior in wild-type ($n = 5$) and TRPV1-null ($n = 4$) mice (see Movie S1). (*B*) Propofol-induced nociception is abolished in TRPA1$^{-/-}$ animals (n = 5); *, $P <$ 1E-6 compared with TRPA1$^{+/-}$ littermates ($n = 5$). (*C* and *D*) Integrated EMG activity from semitendinosus muscle of TRPA1$^{+/-}$ and TRPA1$^{-/-}$ mice after injection of 30 μl of propofol (500 μM) or capsaicin (50 μM, 5 min later) into the femoral artery ($n = 3$ for both).

sites. In contrast, we found that isoflurane (0.9 mM) produced negligible effects on TRPA1 when applied together with an apparent saturating dose of octanol (1.8 mM) (Fig. 4 *C* and *F*). Therefore, these data are consistent with VGAs and alcohols acting at a common site(s) (which reach saturation with submaximal efficacy). Note that this result cannot be explained by an overall "ceiling effect" on channel gating, because the responses to octanol were submaximal (<50% of 1 mM AITC at +200 mV). However, coapplication of propofol and octanol produced an additive response at TRPA1 (Fig. 4 *E* and *F*) suggesting that propofol acts at a distinct site from alcohols and VGAs. To further confirm a common action of alcohols and VGAs we compared the relative ability of these compounds to activate different TRPs. Fig. 4*D* shows that at holding potential of −50 mV, octanol and isoflurane selectively activated TRPA1 with negligible effects at TRPV1 and TRPM8. Thus, octanol and isoflurane exhibit a similar activation profile at TRP channels, consistent with a common mechanism of action.

AITC and several other volatile compounds are electrophiles and can activate TRPA1 by covalent modification of N-terminal cysteines (22, 23). This does not seem to be the case for GAs because their chemical structures do not support such a mechanism. Moreover, in contrast to AITC, we found that successive applications of isoflurane could evoke TRPA1 currents (Fig. S3). However, isoflurane failed to activate TRPA1 after AITC treatment, suggesting that covalent modification renders TRPA1 unresponsive to GAs. AITC similarly depresses activation by voltage (23) and menthol (24), suggesting an allosteric mechanism of inhibition.

Finally, we tested whether GAs activate by altering TRPA1 voltage sensitivity. To avoid the confound of GA-induced pore block, we measured TRPA1 open probability in cell-attached patches. Fig. S2 shows that desflurane shifted the $V_{1/2}$ from 72.1 to 42.5 mV and increased the maximal open probability >6-fold. These observations suggest that, although voltage enhances the activation produced by desflurane, GAs can nonetheless act in a voltage-independent manner.

TRPA1 Mediates Propofol-Induced Pain. Next, we asked whether TRPA1 mediates the well described pain accompanying injections of propofol (2–4). First, we tested whether topical application of propofol could induce nocifensive behaviors. Fig. 5*A* shows that when applied to the nasal epithelium, propofol induced ≈40 s of pain-related behavior (see *Materials and Methods* and Movie S1) over a 2-min period, whereas the vehicle (mineral oil) was without effect. A similar nocifensive response to propofol was seen in TRPV1-null mice (Fig. 5*A*). In contrast, nocifensive behavior was completely absent in TRPA1-null mice (Fig. 5*B*); whereas TRPA1$^{+/-}$ littermates exhibited a robust response of ≈35 s. Second, we tested the effects of propofol in a vascular-pain model by using the flexor reflex response (25). Fig. 5 *C* and *D* shows that propofol, injected into the femoral artery, evoked reflex muscle activity in TRPA1$^{+/-}$ mice but produced no responses in TRPA1-null animals. In contrast, capsaicin produced robust responses in both groups. Thus, taken together these data indicate that TRPA1 is critical for propofol-evoked nociception.

Isoflurane Evokes Greater Neurogenic Inflammation Compared with Sevoflurane. Excitation of sensory nerves can evoke the release of neuropeptides that contribute to inflammation. We therefore asked whether VGAs could modulate this process through their actions at TRPA1. To test this, we applied AITC to the ears of mice—a commonly used model of neurogenic inflammation (26)—and compared the ear swelling when animals were anesthetized either with isoflurane or sevoflurane (1.2 MAC, see *Materials and Methods*). Fig. 6 *A* and *B* shows that AITC induced significantly greater swelling in animals anesthetized with isoflurane at all time points measured (15–120 min, $n = 7$, $P < 0.01$). Isoflurane also caused a small increase in swelling in the unpainted ear at 90 and 120 min. However, this effect did not occur in the absence of AITC when animals were administered isoflurane alone (data not shown), suggesting that it was because of an interaction of isoflurane and

PHARMACOLOGY

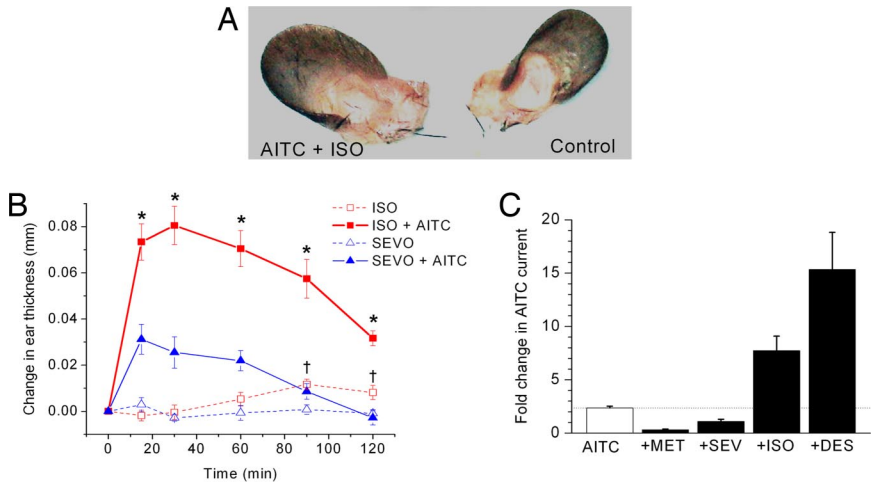

Fig. 6. AITC-induced ear swelling is greater during anesthesia with isoflurane compared with sevoflurane. (*A* and *B*) AITC (0.6%, 20 μl) was applied to one ear of mice and the contralateral ear received mineral oil alone. Animals were anesthetized with 1.2 MAC of isoflurane or sevoflurane for 60 min followed by 60 min of recovery. Data show the change in ear thickness from baseline (both groups, $n = 7$) *, $P < 0.01$, AITC+Isoflurane versus other groups ANOVA; †, $P < 0.05$ for isoflurane alone versus sevoflurane alone. (*C*) Pungent (isoflurane and desflurane) but not smooth (methoxyflurane and sevoflurane) VGAs (0.5–0.65 mM) enhance currents evoked by AITC (10 μM) in TRPA1-expressing oocytes ($n = 3$–4 for each point). *, $P < 0.05$ versus AITC alone.

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. 6*C* 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 $\geq$ isoflurane $\ggg$ sevoflurane/halothane) and this does not correspond with their ability to partition into the membrane (halothane/sevoflurane $\gg$ 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 (C57Bl6/J 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 $MgCl_2$, 1.2 mM $CaCl_2$, 10 mM Hepes, 10 mM glucose, pH 7.3. For cell-attached experiments, NaCl was replaced with KCl in the bath solution. The pipette solution contained 140 mM NaCl or KCl, 10 mM Hepes, 5 mM EGTA, pH 7.3. For neuronal recording NaCl was replaced with K-gluconate (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 mV 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 $NP_o > 750$ ms. Defolliculated *Xenopus laevis* oocytes were injected with ≈10 ng of hTRPA1 (gift of Ardem Patapoutian, The Scripps Research Institute, La Jolla, CA). Oocytes were placed in a Perspex chamber and continuously superfused (5 ml·min^{-1}) with Ca^{2+}-free solution containing 100 mM NaCl, 2.5 mM KCl, 5 mM Hepes, 1 mM $MgCl_2$ and titrated to pH 7.3 with ≈5 mM NaOH.

Ca^{2+} 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 (Compix Inc.).

Behavioral Experiments and Neurogenic Inflammation. 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*$^{-/+}$ and *TRPA1*$^{-/-}$ mice (5–7 weeks). Nocifensive 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 semitendinosus 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 CBJ/A mice (4–6 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 overnight. From 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 enflurane (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.

ACKNOWLEDGMENTS. We thank Tim Hales (George Washington University) and Matt Jones (University of Wisconsin) for discussion and critical comments, Russell Wall (Georgetown University Medical Center) for helpful advice with anesthesia experiments, and Xiangbin Wang for technical assistance. This work was supported by grants from the National Multiple Sclerosis Society and the National Institutes of Health.

1. Hemmings HC, Jr, *et al.* (2005) Emerging molecular mechanisms of general anesthetic action. *Trends Pharmacol Sci* 26:503–510.
2. Tan CH, Onsiong MK (1998) Pain on injection of propofol. *Anaesthesia* 53:468–476.
3. Picard P, Tramer MR (2000) Prevention of pain on injection with propofol: A quantitative systematic review. *Anesth Analg* 90:963–969.
4. Doenicke AW, Roizen MF, Rau J., Kellermann W. Babl J (1996) Reducing pain during propofol injection: The role of the solvent. *Anesth Analg* 82:472–474.
5. MacIver MB, Tanelian DL (1990) Volatile anesthetics excite mammalian nociceptor afferents recorded in vitro. *Anesthesiology* 72:1022–1030.
6. Campbell JN, Raja SN, Meyer RA (1984) Halothane sensitizes cutaneous nociceptors in monkeys. *J Neurophysiol* 52:762–770.
7. Mutoh T, Tsubone H, Nishimura R, Sasaki N (1998) Responses of laryngeal capsaicin-sensitive receptors to volatile anesthetics in anesthetized dogs. *Respir Physiol* 111:113–125.
8. Eger EI, II (1995) New drugs in anesthesia. *Int Anesthesiol Clin* 33:61–80.
9. Kong CF, Chew ST, Ip-Yam PC (2000) Intravenous opioids reduce airway irritation during induction of anaesthesia with desflurane in adults. *Br J Anaesth* 85:364–367.
10. Zhang Y, Eger EI, II, Dutton RC, Sonner JM (2000) Inhaled anesthetics have hyperalgesic effects at 0.1 minimum alveolar anesthetic concentration. *Anesth Analg* 91:462–466.
11. Tomi K, *et al.* (1993) Alterations in pain threshold and psychomotor response associated with subanaesthetic concentrations of inhalation anaesthetics in humans. *Br J Anaesth* 70:684–686.
12. Pingle SC, Matta JA, Ahern GP (2007) Capsaicin receptor: TRPV1 a promiscuous TRP channel. *Handb Exp Pharmacol* 179:155–171.
13. Bautista DM, *et al.* (2006) TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. *Cell* 124:1269–1282.
14. Jordt SE, *et al.* (2004) Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature* 427:260–265.
15. Macpherson LJ, *et al.* (2005) The pungency of garlic: Activation of TRPA1 and TRPV1 in response to allicin. *Curr Biol* 15:929–934.
16. Bautista DM, *et al.* (2005) Pungent products from garlic activate the sensory ion channel TRPA1. *Proc Natl Acad Sci USA* 102:12248–12252.
17. Caterina MJ, *et al.* (2000) Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science* 288:306–313.
18. Davis JB, *et al.* (2000) Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. *Nature* 405:183–187.
19. Kwan KY, *et al.* (2006) TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. *Neuron* 50:277–289.
20. Mascia MP, Trudell JR, Harris RA (2000) Specific binding sites for alcohols and anesthetics on ligand-gated ion channels. *Proc Natl Acad Sci USA* 97:9305–9310.
21. Mihic SJ, *et al.* (1997) Sites of alcohol and volatile anaesthetic action on GABA(A) and glycine receptors. *Nature* 389:385–389.
22. Hinman A, Chuang HH, Bautista DM, Julius D (2006) TRP channel activation by reversible covalent modification. *Proc Natl Acad Sci USA* 103:19564–19568.
23. Macpherson LJ, *et al.* (2007) Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. *Nature* 445:541–545.
24. Karashima Y, *et al.* (2007) Bimodal action of menthol on the transient receptor potential channel TRPA1. *J Neurosci* 27:9874–9884.
25. Ando R, Watanabe C (2005) Characteristics of propofol-evoked vascular pain in anaesthetized rats. *Br J Anaesth* 95:384–392.
26. Inoue H, Asaka T, Nagata N, Koshihara Y (1997) Mechanism of mustard oil-induced skin inflammation in mice. *Eur J Pharmacol* 333:231–240.
27. McNamara CR, *et al.* (2007) TRPA1 mediates formalin-induced pain. *Proc Natl Acad Sci USA* 104:13525–13530.
28. Bandell M, *et al.* (2004) Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron 41:849–857.
29. Macpherson LJ, *et al.* (2007) An ion channel essential for sensing chemical damage. *J Neurosci* 27:11412–11415.
30. Trevisani M, *et al.* (2007) 4-Hydroxynonenal, an endogenous aldehyde, causes pain and neurogenic inflammation through activation of the irritant receptor TRPA1. *Proc Natl Acad Sci USA* 104:13519–13524.
31. Ji RR, Kohno T, Moore KA, Woolf CJ (2003) Central sensitization and LTP: Do pain and memory share similar mechanisms? *Trends Neurosci* 26:696–705.
32. Matta JA, Ahern GP (2007) Voltage is a partial activator of rat thermosensitive TRP channels. *J Physiol* 585:469–482.
33. Franks NP, Lieb WR (1996) Temperature dependence of the potency of volatile general anesthetics: Implications for in vitro experiments. *Anesthesiology* 84:716–720.