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General Anesthetics Sensitize the Capsaicin Receptor Transient Receptor Potential V1

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ABSTRACT

General anesthetics (GAs) are central nervous system depressants that render patients unresponsive to external stimuli. In contrast, many of these agents are also known to stimulate peripheral sensory nerves, raising the possibility that they may exacerbate tissue inflammation. We have found that pungent GAs excite sensory neurons by directly activating the transient receptor potential (TRP) A1 ion channel. Here, we show that GAs also sensitize the capsaicin receptor TRPV1, a key ion channel expressed in nociceptive neurons. Clinically relevant concentrations of isoflurane, sevoflurane, enflurane, and desflurane sensitize TRPV1 to capsaicin and protons and reduce the threshold for heat activation. Furthermore, isoflurane directly activates TRPV1 after stimulation of protein kinase C. Likewise, isoflurane excites TRPV1 and sensory neurons during concomitant application of bradykinin, a key inflammatory mediator formed during tissue injury. Thus, GAs can enhance the activation of TRPV1 that occurs during surgically induced tissue damage. These results support the hypothesis that some GAs, through direct actions at TRP channels, increase postsurgical pain and inflammation.

Volatile general anesthetics (VGAs) are a diverse group of volatile and gaseous substances with the shared ability to suppress CNS activity (Franks, 2008). In addition to hypnosis or unconsciousness, most of these drugs also cause a varying degree of amnesia, muscle relaxation, and blunting of sympathetic responses (Miller et al., 2002). Although the precise molecular mechanisms are unresolved, several studies suggest that GAs can inhibit CNS activity by sensitizing or activating $GABA_A$ receptors (Nakahiro et al., 1991; Wakamori et al., 1991; Jones et al., 1992) and background K^+ channels (Franks and Lieb, 1988; Gray et al., 1998; Patel et al., 1999) or by inhibiting glutamate receptors (Yamakura and Harris, 2000; Hollmann et al., 2001) and presynaptic transmitter release (van Swinderen et al., 1999).

In contrast to their effects in the CNS, several VGAs are known to excite peripheral nociceptive ("pain-sensing") neurons (Mutoh et al., 1998; Mutoh and Tsubone, 2003) and are perceived as pungent. Indeed, inhalation of desflurane [greater than 1 minimum alveolar concentration (MAC)] and isoflurane (greater than 1.5 MAC) produces airway irritation in humans, and therefore, these GAs are avoided as induction agents (Eger, 1995; Kong et al., 2000). We have shown recently that these pungent GAs excite sensory neurons by directly activating the transient receptor potential (TRP) channel TRPA1 (mustard-oil receptor) (Matta et al., 2008). Furthermore, we found that TRPA1-dependent neurogenic inflammation is greater during anesthesia with pungent compared with nonpungent anesthetics. Thus, we have proposed that GAs, by modulating TRPA1, could exacerbate surgical pain and inflammation.

TRPA1 is expressed in 25% of sensory neurons, and it is noteworthy that it is almost always found coexpressed with another pain-sensing ion channel, capsaicin receptor TRPV1 (Bautista et al., 2006). TRPV1 imbues sensory neurons with sensitivity to the plant irritant *N*-(4-hydroxy-3-methoxybenzyl)-8-methylnon-6-eneamide (capsaicin) and is more widely distributed than TRPA1; approximately 50% of dorsal root, nodose, and trigeminal ganglia express TRPV1 based on mRNA or capsaicin sensitivity (Nagy et al., 2004; Kobayashi et al., 2005; Bautista et al., 2006). Targeted disruption of the TRPV1 gene in mice profoundly attenuates thermal hyperalgesia (Caterina et al., 2000; Davis et al., 2000), supporting a

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ABBREVIATIONS: VGA, volatile general anesthetic; GA, general anesthetic; TRP, transient receptor potential; CNS, central nervous system; MAC, minimum alveolar concentration; HEK, human embryonic kidney; PKC, protein kinase C; BK, bradykinin; MES, 4-morpholineethanesulfonic acid; AMG9810, (*E*)-3-(4-*t*-butylphenyl)-*N*-(2,3-dihydrobenzo[*b*][1,4] dioxin-6-yl)acrylamide; capsaicin, *N*-(4-hydroxy-3-methoxybenzyl)-8-methylnon-6-eneamide; capsazepine, *N*-[2-(4-chlorophenyl)ethyl]-1,3,4,5-tetrahydro-7,8-dihydroxy-2-H-2-benzazepine-2-carbothioamide; PDBu, phorbol 12, 13-dibutyrate.

central role for TRPV1 in inflammatory pain. A number of diverse chemical and physical stimuli activate TRPV1, including heat, protons, cations, and inflammatory fatty acids (Pingle et al., 2007). It is typical that these noxious stimuli act in a cooperative or synergistic manner to enhance TRPV1 channel activity. Indeed, protons and ethanol act primarily by sensitizing the channel to other stimuli, as opposed to being direct agonists (Tominaga et al., 1998; Trevisani et al., 2002). We therefore considered the possibility that GAs, although not directly activating TRPV1, could nonetheless sensitize TRPV1. Our results show that clinically relevant concentrations of VGAs sensitize TRPV1 to heat, protons, and capsaicin. Furthermore, inflammatory stimuli, including protein kinase C and bradykinin, enhance the stimulatory effects of VGAs. These results suggest that VGAs, by their collective actions at TRPA1 and TRPV1 ion channels, may exacerbate peripheral nociceptive signaling in the context of surgery.

Materials and Methods

HEK Cell and Sensory Neuron Electrophysiology. HEK293F cells (Invitrogen, Carlsbad, CA) were cultured in Dulbecco's modified Eagle's medium supplemented with 1% nonessential amino acids and 10% fetal calf serum. Cell cultures were maintained at 37°C with 5% CO_2. Cells were transfected with rat TRPV1 (a gift of David Julius), and green fluorescent protein cDNA using Lipofectamine Transfection Reagent (Invitrogen) and were used 24 to 48 h after transfection. Nodose ganglia were obtained from adult mice (C57BL6/J and TRPV1-null), cut, digested with collagenase, and cultured in Neurobasal + 2% B-27 medium (Invitrogen), 0.1% L-glutamine, and 1% penicillin/streptomycin on poly(D-lysine)-coated glass coverslips at 37°C in 5% CO_2. Neurons were used within 24 to 36 h of culture. Whole-cell and single-channel patch-clamp recordings were performed using an EPC8 amplifier (HEKA, Lambrecht/Pfalz, Germany). The current signal was low pass-filtered at 1 to 3 kHz and sampled at 4 kHz. Currents were further filtered for display purposes. For whole-cell and excised patch recordings, the bath

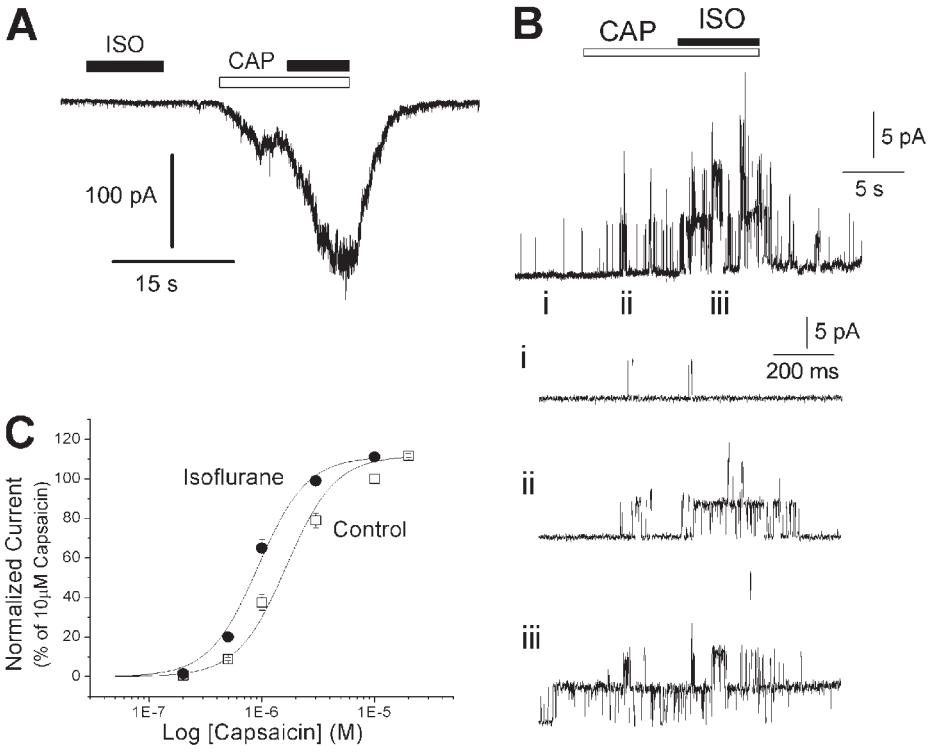

Fig. 1. Isoflurane enhances capsaicin-evoked TRPV1 currents. A, representative current trace from a voltage-clamped neuron treated sequentially with isoflurane (0.9 mM), capsaicin (30 nM), and isoflurane plus capsaicin. B, top, continuous recording of capsaicin-sensitive channels in an outside-out patch (holding potential, +50 mV) in the presence of capsaicin (30 nM) or capsaicin plus isoflurane (0.9 mM). Bottom (i–iii), expanded sections of recording from indicated time points. C, dose-response curves in oocytes for activation of TRPV1 by capsaicin with or without isoflurane (0.9 mM, n = 3–7 for each data point). The smooth curves are fits to a Hill function yielding EC_{50} values of 1.64 ± 0.12 and 0.81 ± 0.04 μM for control and isoflurane, respectively.

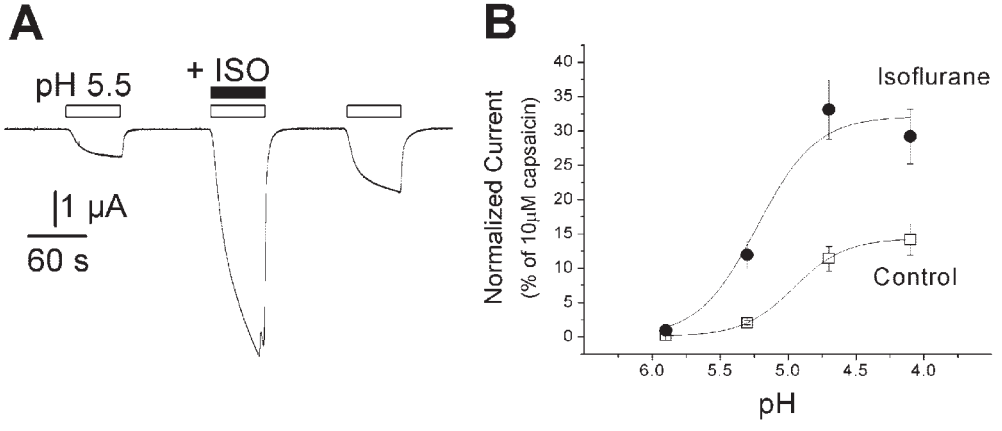

Fig. 2. Isoflurane enhances the sensitivity of TRPV1 to protons. A, current trace from a TRPV1-expressing oocyte treated with pH 5.5 and pH 5.5 plus isoflurane (0.9 mM) solutions. B, dose-response curves in oocytes for activation of TRPV1 by protons in the absence or presence of isoflurane (0.9 mM, n = 3–7 for each data point). The smooth curves are fits to a Hill function yielding a pEC_{50} value of 4.95 ± 0.15 and 5.23 ± 0.10 for control and isoflurane, respectively. Isoflurane also increased the maximal response from 14.3 to 32.2% of 10 μM capsaicin ($P < 0.01$).

solution contained 140 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 1 mM EGTA, 10 mM HEPES, and 10 mM glucose, pH 7.3 (290 mOsmol). The pipette solution contained 140 mM CsCl or KCl, 10 mM NaCl, 10 mM HEPES, 5 mM EGTA, 2 mM MgATP, and 0.03 mM GTP, pH 7.3. The peak amplitudes measured during either the prepulse or the tail current (within 1 ms) were plotted as a function of the test potential and were normalized to the maximal current obtained from the following Boltzmann function: $I_{Tail} = \{(I_{max} - I_{min})/[1 + \exp((V - V_{1/2})/s)]\} + I_{min}$, where $V_{1/2}$ is the potential that elicits half-maximal ctivation, s is the slope factor, and I_{min} is the minimum current observed.

Oocyte Electrophysiology. Defolliculated *Xenopus laevis* oocytes (harvested from adult females anesthetized with 0.5 g/l tricaine methanesulfonate) were injected with ~10 ng of wild-type rat TRPV1 cRNA or mutant S502A/S800A TRPV1 cRNA (a gift of Makoto Tominaga). Oocytes were placed in a Perspex chamber and continuously superfused (5 ml/min) with Ca^{2+}-free solution containing 100 mM NaCl, 2.5 mM KCl, 5 mM HEPES, and 1 mM $MgCl_2$ and titrated to pH 7.3 with ~5 mM NaOH. For solutions with pH value of less than 6.0, HEPES was replaced with either 5 mM MES or 5 mM sodium citrate. Oocytes were routinely voltage-clamped at −60 mV at 22 to 23°C. For heat activation, bath temperature was increased from ~22 to 50°C over ~100 s using an inline solution heater (Warner Instruments, Hamden, CT). The temperature was monitored continuously with a probe placed within 2 mm of the oocyte. The temperature-activation threshold was defined as a 20% increase in current above baseline.

Volatile General Anesthetics. Saturated stock solutions of VGAs were prepared in gas-tight bottles by dissolving excess anesthetic agents in bath solutions and stirring vigorously overnight. From these stock solutions, fresh dilutions were made up every 40 to 60 min. Concentrations of volatile anesthetics in the bath solutions were verified using a modified head-space gas chromatography method. The gas chromatograph (Carlo Erba, Milan, Italy) was equipped with a flame ionization detector and mass spectrometer. Standards were prepared from a mixture of halothane, isoflurane, and sevoflurane dissolved in acetonitrile with enflurane as an internal standard. The equivalent MACs were calculated using published conversion factors reported for halothane (1 MAC, 0.27 mM), isoflurane (1 MAC, 0.31 mM), and sevoflurane (1 MAC, 0.35 mM) in rat at 37°C (Franks and Lieb, 1996).

Chemicals. Capsazepine, phorbol 12, 13-dibutyrate (PDBu), bradykinin, and staurosporine were obtained from Sigma (St. Louis, MO). Capsaicin and AMG9810 were purchased from Tocris Cookson (Ellisville, MO). Drugs were prepared as stock solutions in dimethyl sulfoxide or ethanol and diluted into physiological solution before experiments.

Statistical Analysis. Data are given as mean ± S.E.M., and statistical significance was evaluated using analysis of variance or Student's t test.

Results

Volatile Anesthetics Sensitize TRPV1 to Capsaicin and Protons. Although our previous data showed that VGAs do not directly activate TRPV1, we considered the possibility that they could nonetheless sensitize TRPV1 to other modes of activation. Indeed, a diverse array of physical and chemical stimuli activate TRPV1 (Pingle et al., 2007), and these stimuli produce synergistic effects when applied together. In sensory neurons, isoflurane (0.9 mM or ~2.9 MAC) enhanced by approximately 3-fold whole-cell currents evoked by capsaicin (30 nM; Fig. 1A, $n = 5$). Furthermore, isoflurane increased capsaicin-evoked single channel activity in cell-free, outside-out patches (Fig. 1B). We found that VGAs produced a similar sensitization of TRPV1 to protons. In TRPV1-expressing oocytes, isoflurane (0.9 mM) significantly enhanced by approximately 10-fold the currents evoked by a pH 5.5 solution (Fig. 2A, $n = 4$). Dose-response analyses show that isoflurane reduced the half-maximal concentration required for activation by capsaicin and protons (Figs. 1C and 2B); the capsaicin EC_{50} was reduced from ~1.6 to 0.8 μM ($P < 0.01$), and the proton pEC_{50} was increased from 4.95 to 5.23 ($P < 0.01$). In addition, isoflurane enhanced the maximal proton-evoked current by ~3 fold.

Anesthetics Enhance Voltage and Thermal Sensitivity of TRPV1. TRPV1 is a voltage-sensitive channel; membrane depolarization gates TRPV1, and half-maximal activa-

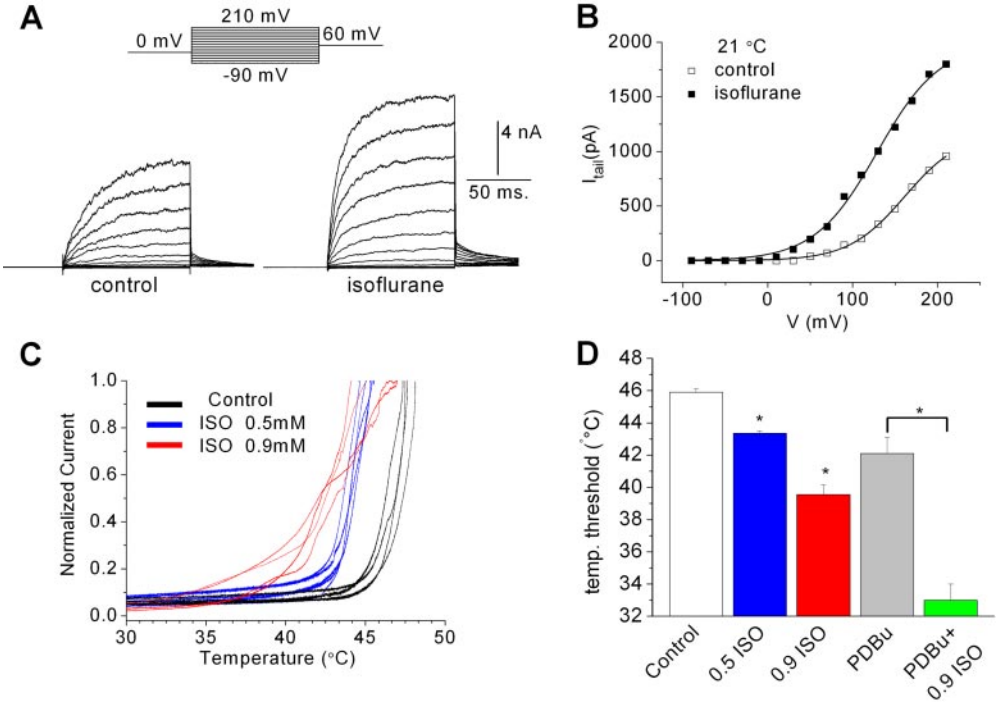

Fig. 3. Volatile GAs increase the sensitivity of TRPV1 to voltage and heat. A, TRPV1 currents activated by a family of voltage steps (−90 to 210 mV) under control conditions and with isoflurane (0.9 mM). B, plots of tail current versus voltage prepulse for control and isoflurane. Smooth lines are best fits to a Boltzmann function yielding $V_{1/2}$ values of 161.5 ± 2.7 and 129.0 ± 3.6 mV. C, current versus temperature plots in TRPV1-expressing oocytes for control (black), 0.5 mM (blue), or 0.9 mM (red) isoflurane. Currents are normalized to the maximum current evoked at 47°C. D, mean thresholds of heat activation for control, 0.5 mM isoflurane, 0.9 mM isoflurane, PDBu (200 nM, 3 min), and PDBu + isoflurane (0.9 mm), *, $P <$ 0.01 compared with control or versus PDBu alone. Data are the mean of four to five oocytes.

tion ($V_{1/2}$) is seen at ~120 mV (at 25°C) (Voets et al., 2004). Although, these membrane potentials are supraphysiological, agonists enhance the sensitivity of TRPV1 to voltage such that the channel responds to voltage in the physiological range. In addition, agonists increase the maximal voltage-evoked current (Matta and Ahern, 2007). Likewise, we found that application of isoflurane (0.9 mM) enhanced the currents evoked by depolarization in HEK293 cells expressing TRPV1 (Fig. 3A). Figure 3B shows the Boltzmann fits to these data. Isoflurane reduced the $V_{1/2}$ by 23.0 ± 6.2 mV and enhanced the maximal current by 15 ± 6% (n = 5).

TRPV1 is characteristically gated by heat with an activation threshold of ~42 to 43°C in mammalian cells and ~46°C in oocytes (Caterina et al., 1997). We asked whether isoflurane could alter this temperature sensitivity. In TRPV1-expressing oocytes, isoflurane significantly reduced the temperature threshold in a dose-dependent manner (Fig. 3, C and D); the thresholds for control, 0.5 and 0.9 mM isoflurane, respectively, were ~46, 43, and 40°C.

Clinical Concentrations of Diverse VGAs Regulate TRPV1. Next, we explored whether VGAs could effectively modulate TRPV1 at clinically relevant concentrations. Figure 4 shows that isoflurane (0.1–2 mM) enhanced proton-evoked responses in a dose-dependent manner, and a significant potentiation occurred between 0.1 to 0.9 mM (corresponding to ~0.3–3 MAC). Thus isoflurane, at concentrations achieved during maintenance anesthesia, is capable of enhancing TRPV1 activity. Next, we explored the effects of different VGAs. Figure 5 shows that VGAs (0.6 mM) with the most pungency, desflurane and enflurane, enhanced proton-evoked currents significantly more than the less pungent agents isoflurane and sevoflurane. Therefore, similar to our earlier data with TRPA1 (Matta et al., 2008), there is a correlation, albeit less pronounced, between VGA pungency and TRPV1 sensitization.

Protein Kinase C and Bradykinin Enhance VGA Activation of TRPV1. Many inflammatory mediators engage G-protein coupled receptors expressed on sensory neurons, leading to the activation of protein kinase C (PKC). In turn, PKC produces a marked sensitization of TRPV1 (Premkumar and Ahern, 2000; Vellani et al., 2001; Numazaki et al., 2002). We found that PKC significantly enhanced heat activation of TRPV1 by VGAs. After application of PDBu, isoflurane (0.9 mM) reduced the temperature threshold further from 45.9 ± 0.2 to 32.8 ± 0.8°C, whereas PDBu alone reduced it to 39.3 ± 2.3°C (Fig. 3D). In contrast, PDBu did not produce a significant effect in oocytes expressing mutant TRPV1 receptors that lack essential PKC phosphorylation sites [S502A/S800A, 39.4 ± 1.9°C and 37.3 ± 0.7°C for isoflurane (n = 5) and PDBu + isoflurane (n = 3), respectively], indicating that PDBu produces its effects through direct phosphorylation of TRPV1. This effect of PKC was more dramatic in mammalian cells. After PDBu treatment, VGAs (0.9 mM) evoked inward currents at room temperature (25°C) in both TRPV1-express-

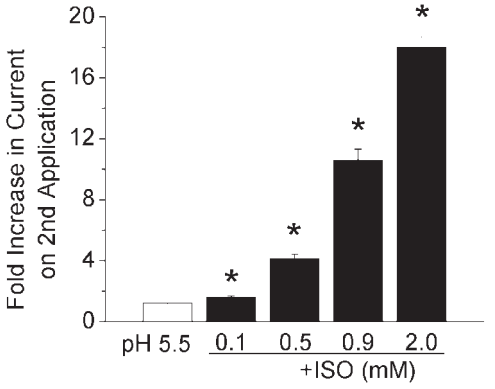

Fig. 4. Isoflurane modulates TRPV1 at clinically relevant concentrations. The mean -fold increase in proton-evoked currents in oocytes produced by coapplication with varying concentrations of isoflurane (0.1–2 mM, 0.3–3 MAC) or a second application of pH 5.5 alone. Data are the mean of three to four oocytes; *, $P < 0.01$ compared with pH 5.5 alone.

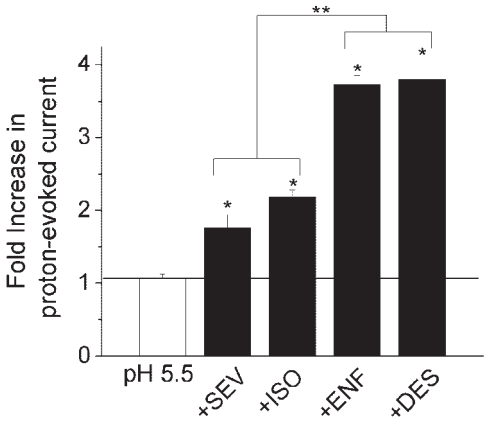

Fig. 5. Modulation of TRPV1 by diverse volatile anesthetics. The relative potentiation of proton (pH 5.5)-evoked currents by 0.6 mM concentrations of sevoflurane, isoflurane, enflurane, and desflurane (n = 3–4 for each data point). *, $P < 0.01$ compared with control. **, $P < 0.01$ between designated groups of VGAs (one-way analysis of variance).

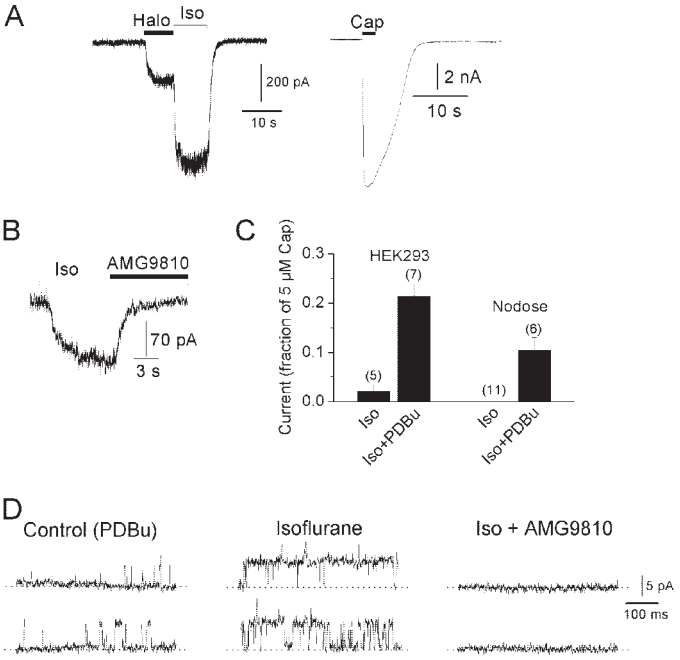

Fig. 6. Isoflurane activates TRPV1 in a PKC-dependent manner. A, after pretreatment with PDBu (500 nM), halothane (0.9 mM) and isoflurane (0.9 mM) activate currents in TRPV1-expressing HEK293 cells. B, AMG9810 (1 μM) inhibits the current evoked by isoflurane (0.9 mM) in a sensory neuron (pretreated with PDBu). C, mean current evoked by isoflurane in TRPV1-expressing HEK293 cells and capsaicin-sensitive sensory neurons with or without PDBu treatment. Data are normalized to responses evoked by a saturating capsaicin concentration (5 μM), and the number of cells is given in parentheses. D, single TRPV1 channel activity in an outside-out patch from a sensory neuron in response to isoflurane (0.9 mM) and AMG9810 (1 μM). The holding potential was +60 mV.

ing HEK293 cells (Fig. 6, A and C, $n = 7$) and in capsaicin-sensitive sensory neurons (Fig. 6, B–D, $n = 6$). Furthermore, isoflurane activated single TRPV1 channel activity in neurons after PDBu treatment. These responses were completely inhibited by the TRPV1-specific antagonist AMG9810 (Fig. 6, B and D, $n = 3$), indicating the selective activation of TRPV1.

Surgery is associated with tissue injury and the release of numerous inflammatory mediators that can activate/sensitize sensory neurons. One key "pain" signaling molecule is bradykinin (BK). BK acting through its type 2 receptor can activate/sensitize TRPV1 (Cesare and McNaughton, 1996; Premkumar and Ahern, 2000; Chuang et al., 2001; Shin et al., 2002) and TRPA1 (Bandell et al., 2004; Bautista et al., 2006) via multiple signaling pathways. We found that BK enhanced the responses of sensory neurons to isoflurane (Fig. 7, C and D). Under control conditions, isoflurane produced negligible responses, but after BK treatment, there was a marked increase in current ($n = 7$, $P < 0.01$). These neurons were all insensitive to allyl isothiocyanate, excluding a contribution of TRPA1 and the TRPV1 blocker *N*-[2-(4-chlorophenyl)ethyl]-1,3,4,5-tetrahydro-7,8-dihydroxy-2-H-2-benzazepine-2-carbothioamide (capsazepine, 1 μM), completely inhibited responses to isoflurane (in three of three cells), indicating that the major effect of BK was to recruit previously quiescent TRPV1 channels. These responses are sufficient to drive membrane excitability; under current clamp, coapplication of isoflurane and BK depolarized neurons and initiated sustained spike discharge (Fig. 7D, $n = 3$). Taken together, these data provide strong support for the hypothesis that tissue injury can amplify the excitatory actions of VGAs on sensory neurons.

Volatile Anesthetics Interact Directly with TRPV1 Channels. VGAS could potentially alter TRPV1 activity by altering cellular signaling cascades. However, our data showed that VGAs retained their effect on TRPV1 in cell-free patches, indicating a membrane-delimited effect. To examine whether VGAs regulate TRPV1 by directly interacting with the TRPV1 protein, as opposed to effects on membrane fluidity, we investigated the action of long-chain alcohols. The results of several studies indicate that VGAs and alcohols bind directly to $GABA_A$ and glycine receptors at a common binding site located between transmembrane domains 2 and 3 (Mihic et al., 1997; Mascia et al., 2000). Furthermore, alcohols exhibit 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 (Mihic et al., 1997; Mascia et al., 2000). These data are consistent with alcohols binding to a "pocket" on these channels of finite molecular volume. Figure 8, A to D, shows that *n*-alcohols (2–12 carbons) enhanced voltage-dependent activation of TRPV1 in proportion to carbon chain length. Shifts in the $V_{1/2}$ value and increases in

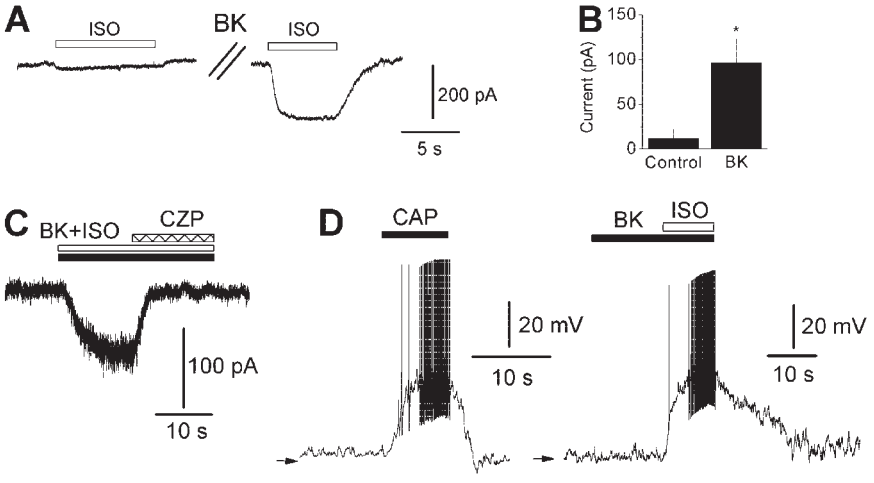

Fig. 7. Isoflurane and bradykinin synergistically excite TRPV1 and sensory neurons. A, BK (10 μM) enhances capsaicin (30 nM)-evoked currents in sensory neurons. B and C, coapplication of BK and isoflurane (0.9 mM) induces inward currents in sensory neurons, and these currents are inhibited by capsazepine (1 μM). D, coapplication of BK and isoflurane depolarizes a capsaicin-sensitive sensory neuron under current clamp. The arrow indicates −60 mV.

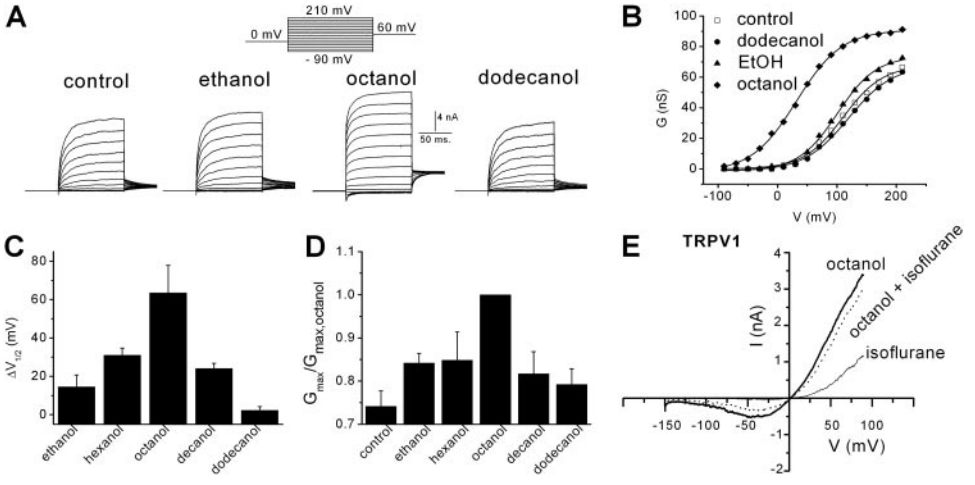

Fig. 8. Volatile anesthetics interact directly with TRPV1. A, representative TRPV1 current traces in response to voltage steps in the presence of various alcohols. B, Boltzmann fits to the conductance measured at the end of test potential. C and D, summary of changes in TRPV1 $V_{1/2}$ and maximal conductance induced by alcohols ($n = 4$–7 cells). Concentrations were the following: ethanol (508 mM), hexanol (3 mM), octanol (1 mM), decanol (0.6 mM), and dodecanol (0.1 mM) and were chosen according to the solubility limitations of these alcohols as described previously (Peoples and Weight, 1995). E, Octanol (1.8 mM) and isoflurane (0.9 mM) modulate TRPV1 in a nonadditive fashion.

maximal conductance reached a maximum with octanol; thereafter, decanol produced a smaller response, and dodecanol was without effect. Next, we examined whether alcohols and VGAs act at similar binding site(s) on TRPV1. We found that isoflurane (0.9 mM) produced negligible effects on TRPV1 when applied together with an apparent saturating dose of octanol (1.8 mM) (Fig. 8E). This result was not due to a "ceiling effect," because the responses to octanol were submaximal (~40% of that produced by 10 μM capsaicin at 200 mV; data not shown). Therefore, these nonadditive effects are consistent with VGAs and alcohols acting at the same site(s).

Discussion

GAs generally suppress cell excitability in the CNS and this may occur through a concerted action on many targets, including sensitization/activation of K^+ channels (Franks and Lieb, 1988; Gray et al., 1998; Patel et al., 1999) and GABA receptors (Nakahiro et al., 1991; Wakamori et al., 1991; Jones et al., 1992) and inhibition of *N*-methyl-D-aspartate receptors (Yamakura and Harris, 2000; Hollmann et al., 2001). Therefore, it may seem counterintuitive that GAs can excite peripheral sensory neurons. However, there is abundant evidence that GAs can activate C-fibers innervating the skin (Campbell et al., 1984), cornea (MacIver and Tanelian, 1990), airways (Mutoh et al., 1998; Mutoh and Tsubone, 2003), and vasculature (Doenicke et al., 1996; Tan and Onsiong, 1998; Picard and Tramèr, 2000). Our recent data show that the TRPA1 ion channel mediates these acute noxious effects of GAs (Matta et al., 2008). Our results here show that GAs also sensitize TRPV1, a principal nociceptive ion channel highly expressed in sensory nerves. VGAs at pharmacological concentrations sensitize TRPV1 to activation by various stimuli, including capsaicin, protons, heat, and voltage. It is significant that this effect is greater with pungent compared with nonpungent VGAs. Furthermore, VGAs have a more marked effect under inflammatory signaling conditions; after stimulation of PKC, isoflurane alone activates TRPV1 at room temperature. Likewise, isoflurane directly activates TRPV1 and excites sensory neurons after treatment with the inflammatory mediator bradykinin.

VGAs seem to regulate TRPV1 directly, and several lines of evidence support this idea. First, VGAs were effective in cell-free patches, indicating a membrane-delimited action. Second, although VGAs can alter membrane fluidity, this effect is unlikely to explain their modulating TRPV1; we have observed that treatment with an agent that increases membrane fluidity, Triton X, produces the opposite effect to isoflurane on TRPV1 function (Matta et al., 2007). In addition, the differential modulation of TRPV1 by smooth versus pungent VGAs does not correlate with their lipid solubility, arguing against nonspecific effects on the membrane. Third, long-chain alcohols replicated the effects of VGAs and exhibited a carbon chain-length cutoff, consistent with these agents binding directly to a protein site. Alcohols and VGAs similarly modulate the TRPA1 channel (Matta et al., 2008). Taken together, these results suggest that VGAs interact directly with the TRPV1 protein. The precise molecular sites(s) are unclear. In addition to VGAs, several other ligands (e.g., polyunsaturated fatty acids and 2-aminoethoxydiphenyl borate) are capable of activating both TRPV1 and TRPA1. Thus, it is possible that VGAs interact with common binding sites(s) on these channels. It is interesting that local anesthetics have been shown recently to activate and sensitize TRPV1 (Leffler et al., 2008). This effect is attenuated in TRPV1 mutants lacking capsaicin sensitivity, suggesting that local anesthetics interact, in part, at capsaicin binding sites. In the future, studies with mutagenesis and TRP chimeric proteins may prove useful in identifying potential anesthetic binding sites(s). This approach was used in a recent study of the two-pore K^+ channel, twik-like acid-sensitive K^+, to reveal an essential role for a single amino acid (Met159) in the third transmembrane domain; mutation of this residue abolished anesthetic activation (Andres-Enguix et al., 2007).

Our finding that VGAs can activate TRPV1 during inflammatory signaling has significant implications. First, in the laboratory setting, studies of central sensitization in the spinal cord are routinely performed on anesthetized animals. The use of VGAs in these experiments and subsequent facilitation of TRPV1 (and TRPA1) signaling may influence these measurements. Second, in the clinical setting, our data support the hypothesis that VGAs may augment nociceptive signaling arising from surgical insults (Fig. 9). Tissue injury leads to the release of an array of inflammatory mediators, including ATP, protons, serotonin, bradykinin, and prostaglandins. Protons directly regulate TRPV1. The other mediators act at G-protein-coupled receptors, leading to the formation of arachidonic acid metabolites that can gate TRPV1 (Hwang et al., 2000), and/or the stimulation of protein kinases. PKC-mediated phosphorylation, in particular, profoundly sensitizes TRPV1 to chemical and thermal activation (Premkumar and Ahern, 2000; Vellani et al., 2001; Sugiura et al., 2002). Our data show that PKC stimulation enhances the effects of isoflurane, leading to the activation of TRPV1 at room temperature. Moreover, we show that isoflurane acts in a synergistic fashion with the inflammatory mediator bradykinin to activate TRPV1 currents. Bradykinin signaling also activates TRPA1, and this may occur downstream of TRPV1 activation (Bautista et al., 2006). It is noteworthy that these results suggest that VGAs could enhance the activation of TRPV1 and TRPA1 in the context of tissue injury.

The activation/sensitization of TRPV1 by VGAs has two broad consequences. First, it may lead to increased release of neuropeptides from peripheral terminals, culminating in

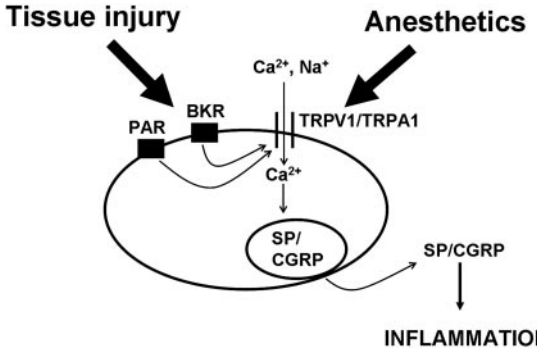

Fig. 9. Model of synergistic activation of TRPs by anesthetics and inflammatory mediators in sensory nerves. Tissue injury leads to accumulation of inflammatory mediators such as proteases and bradykinin, which engage their respective G-protein-coupled receptors (protease receptor, PAR; bradykinin receptor, BKR) expressed on sensory nerves. In turn, this leads to sensitization of TRPV1 and TRPA1 via phospholipase C-dependent pathways. VGAs act directly on TRPs to further enhance their activity. Finally, depolarization and Ca^{2+} entry via TRPs evokes release of inflammatory peptides, including substance P (SP) and calcitonin gene-related peptide (CGRP).

greater neurogenic inflammation (Fig. 9). Indeed, we have shown that mustard oil (TRPA1-dependent)-evoked inflammation is greater in animals anesthetized with pungent compared with nonpungent VGAs (Matta et al., 2008). The higher expression of TRPV1 in sensory nerves suggests that VGAs acting at TRPV1 would produce even more marked effects. Second, it should lead to enhanced C-fiber discharge. Because central sensitization is dependent on C-fiber spike frequency (Ji et al., 2003), then stimulation of TRPV1 by VGAs could lead to a medium to long-lasting facilitation of nociceptive processing in the spinal cord. Thus, through these TRP-dependent mechanisms, VGAs could augment postsurgical pain and inflammation.

How could one avoid the sensitization of TRPs by VGAs? One strategy is through the use of nonpungent agents such as sevoflurane; these nonpungent VGAs have no activity at TRPA1 (Matta et al., 2008) and significantly less activity at TRPV1 compared with pungent VGAs. Another strategy is the use of preemptive analgesia with local anesthetics and opioids (Wilder-Smith, 2000; Gottschalk et al., 2002). However, although these drugs would inhibit C-fiber discharge and transmission in the spinal cord, they would not prevent direct activation of TRPV1 and TRPA1 and the resultant neuropeptide secretion at nerve terminals. Finally, the use of selective TRP antagonists may have usefulness by inhibiting the sensitizing effects of GAs and the generalized excitation of nociceptors by inflammatory mediators.

In summary, our results show that clinically relevant concentrations of volatile anesthetics activate and sensitize the TRPV1 channel. These results suggest that these VGAs may enhance peripheral nociceptive signaling in the context of surgery.

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