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Inhalation anaesthetic isoflurane inhibits the muscarinic cation current and carbachol-induced gastrointestinal smooth muscle contractions

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Abstract

Gastrointestinal tract motility may be demoted significantly after surgery operations at least in part due to anaesthetic agents, but there is no comprehensive explanation of the molecular mechanism(s) of such adverse effects. Anesthetics are known to interact with various receptors and ion channels including several subtypes of transient receptor potential (TRP) channels. Two members of the canonical subfamily of TRP channels (TRPC), TRPC4 and TRPC6 are Ca²⁺-permeable cation channels involved in visceral smooth muscle contractility induced by acetylcholine, the primary excitatory neurotransmitter in the gut. In the present study, we aimed to study the effect of anesthetics on muscarinic receptor-mediated excitation and contraction of intestinal smooth muscle. Here we show that muscarinic cation current (mI_{CAT}) mediated by TRPC4 and TRPC6 channels in mouse ileal myocytes was strongly inhibited by isoflurane (0.5mM), one of the most commonly used inhalation anaesthetics. Carbachol-activated mI_{CAT} was reduced by 63 ± 11% (n=5), while GTPγS-induced (to bypass muscarinic receptors) current was inhibited by 44 ± 9% (n=6). Furthermore, carbachol-induced ileum and colon contractions were inhibited by isoflurane by about 30%. We discuss the main sites of isoflurane action, which appear to be G-proteins and muscarinic receptors, rather than TRPC4/6 channels. These results contribute

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Conflict of interest

The authors have no conflict of interest

to our better understanding of the signalling pathways affected by inhalation anaesthetics, which may cause ileus, and thus may be important for the development of novel treatment strategies during postoperative recovery.

Keywords

G-proteins; GTP γ S (PubChem CID: 37792); TRP channels; carbachol (PubChem CID: 5831); gastrointestinal smooth muscles; general anaesthetics; isoflurane; isoflurane (PubChem CID: 3763); muscarinic receptors

1. Introduction

Despite the efficiency of modern inhalation anesthetics, their mechanism of action remains incompletely characterised. Most importantly, these drugs show a number of adverse reactions, in particular of the gastrointestinal tract (1-3). Indeed, it is well known that both opioids and volatile anaesthetics induce disruption of normal gastrointestinal motility, e.g. paralytic ileus commonly occurs in a postoperative state as one of gastrointestinal complications. The usefulness of the anticholinesterases in these conditions suggests that disruption of the cholinergic control of gut motility may be relevant (4), but the exact molecular targets of isoflurane ((2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane) with relevance to gastrointestinal smooth muscle function remain largely unknown.

In this context, it should be noted that inhalation anesthetics can interact with multiple targets including membrane lipid bilayer and membrane proteins. We have previously extensively characterised the molecular components and signal transduction pathways involved in the cholinergic excitation and contraction of intestinal smooth muscles (5-7). Briefly, acetylcholine, the major excitatory neurotransmitter released by enteric motor neurones, activates the M₂ and M₃ subtypes of muscarinic receptors, the main receptor subtypes present in various visceral smooth muscles. Cholinergic excitation results from the opening of cation TRPC4 and TRPC6 channels, of which TRPC4 is the main contributor (8). TRPC4 activity is controlled in synergy by the M₂/Gi/o and M₃/Gq/PLC signalling pathways, as well as by a concomitant rise in intracellular free Ca²⁺ concentration (9-13). The resulting membrane depolarisation causes activation of L-type Ca²⁺ channels, which, together with InsP₃-induced Ca²⁺ release, provide the major source of Ca²⁺ for the contractile response.

In such a complex system, disruption of muscarinic signalling may occur at multiple levels, from muscarinic receptors, G-proteins and ion channels to Ca²⁺ metabolism and contractile apparatus. Indeed, isoflurane was shown to inhibit muscarinic receptors (14-16), to disrupt muscarinic receptor - G protein coupling (16-18) and to inhibit gastrointestinal motility in rats following even a brief exposure (19). In addition, there is accumulating evidence that inhalation anesthetics act on several TRP channels, such as TRPV1, TRPA1 and TRPM8 (20-22), although canonical TRPC channels have not been yet investigated in this respect. It should be noted that sevoflurane at clinically relevant concentrations strongly inhibited thapsigargin-induced current in ventricular myocytes. Since the current reversed at about 0

mV and it could be inhibited by 2-APB the authors implicated a TRPC channel, such as TRPC1, as the likely sevoflurane target.

Thus we aimed to investigate the effects of isoflurane on the muscarinic cation current (mI_{CAT}) in mouse ileal myocytes attempting to distinguish between its possible molecular targets. To this end, we recorded mI_{CAT} under conditions of “intracellular calcium clamp” to avoid any complications due to altered Ca^{2+} signalling, and compared mI_{CAT} evoked by muscarinic receptor activation with carbachol or, bypassing receptors, by intracellular infusion of GTP γ S in order to activate G-proteins directly.

2. Material and methods

2.1. Tissue preparation

All experimental procedures involving animals were approved by the Animal Studies Committee at Washington University School of Medicine, and were in accordance with the guidelines provided by the National Institute of Health. C57/BL6 wild type male mice (75–90 days old, 25–30 g weight) were euthanized with CO₂ asphyxiation followed by cervical dislocation. For electrophysiological recordings the abdominal cavity was dissected and longitudinal smooth muscles of the ileum was rapidly removed and placed in modified Krebs-HEPES solution containing (in mM) 120 NaCl, 6 KCl, 2.5 CaCl₂, 1.2 MgCl₂, 12 glucose, 10 HEPES, pH adjusted to 7.4 with NaOH.

2.2. Smooth muscle cell isolation

Smooth muscle strips were cut into small segments (about 1 mm in length) in Ca²⁺-free Krebs-HEPES solution. The pieces were digested at 36.5 °C for 17 min in 2 ml of this solution containing collagenase type 1 A (1 mg/ml), soybean trypsin inhibitor (1 mg/ml) and bovine serum albumin (2 mg/ml), and then washed two times with Ca²⁺-free Krebs-HEPES solution. Tissue pieces were then gently triturated with glass Pasteur pipette in Ca²⁺-free Krebs-HEPES solution at room temperature (22–25 °C) to release single myocytes. Cells were placed on glass cover slips in low-Ca²⁺ (0.8 mM) Krebs-HEPES solution and kept at 4 °C until use in experiments for 7 hours after isolation.

All chemicals and reagents were purchased from Sigma Chemical (St. Louis, MO, USA).

2.3. Intact tissue isometric force measurement

Whole thickness colon or ileum segments (1 cm long) were obtained as described above and mounted in 25-ml organ baths filled with Krebs-Ringer solution (composition in mM: 120 NaCl, 6 KCl, 1.2 MgCl₂, 1.35 NaH₂PO₄, 14.4 NaHCO₃, 2.5 CaCl₂, 11.5 glucose) gassed with 5% CO₂ – 95% O₂ mixture at 37 °C. After a 30 min equilibration period, the isometric force was monitored by an external force displacement transducer (World Precision Instruments, Sarasota, FL, USA) connected to a PowerLab data acquisition system (ADInstruments, Colorado Springs, CO, USA). Drugs were added to the bath directly using their concentrated stock solutions prepared using Krebs-Ringer solution.

2.4. Electrophysiology

Transmembrane currents were recorded in the whole-cell configuration of the patch-clamp techniques using an EPC-10 amplifier (HEKA Elektronik Dr.Schulze GmbH, Lambrecht, Germany) at room temperature (22–24 °C) with the pClamp software (Molecular Devices, Sunnyvale, CA, USA). Patch pipettes, pulled from borosilicate glass (1.5 mm OD, 0.86 mm ID; Sutter Instruments, Novato, CA, USA) using a Sutter P-97 micropipette puller (Sutter Instrument, Novato, CA, USA) had a resistance of 4–7 MOhm when filled with the intracellular solution. For mI_{CAT} recordings the pipette solution contained (mM): CsCl 80, MgATP 1, creatine 5, glucose 5, BAPTA 10, HEPES 10, $CaCl_2$ 4.6 (thus “clamping” free Ca^{2+} concentration at 100 nM), pH adjusted to 7.4 with CsOH.

Before current recordings cells were kept in normal Krebs-HEPES bath solution. For recordings of mI_{CAT} , which was induced either by external carbachol (50 μ M) or intracellular GTP γ S (200 μ M) added to the pipette (which activates G-proteins directly, i.e. bypassing the muscarinic receptors), external solution contained (mM): CsCl 120, glucose 12, HEPES 10, pH adjusted to 7.4 with CsOH. When carbachol-induced mI_{CAT} was recorded GTP at 1 mM was added to the pipette solution in order to avoid current desensitization (23). All reagents were purchased from Sigma unless otherwise stated.

After the formation of tight contact between the membrane and pipette, series resistance was compensated by ~ 30%. Holding potential was –40 mV. The steady-state current-voltage (I-V) relationships were measured by slow (6 s duration) voltage ramps from 80 mV to –120 mV, which were applied every 30 s. mI_{CAT} voltage-dependent deactivation kinetics was assessed by stepping the membrane potential from –40 to –120 mV for 1.2 s prior to the voltage ramp. Membrane currents were filtered at 2 kHz and sampled at 10 kHz.

2.5. Data and statistical analysis

Recordings were analyzed and plotted using Clampfit 10 (Molecular Devices, Sunnyvale, CA, USA) and Origin 9.4 software (OriginLab Corporation, Northampton, MA, USA). Data are represented as means $\pm$ SEM (n – number of cells or preparations examined) and differences between groups were evaluated using Student's *t*-test (for two groups) or one-way analysis of variance (ANOVA) (for three groups) followed by a post hoc Tukey-Kramer multiple comparison test to compare all data. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Isoflurane inhibits carbachol-induced mI_{CAT} in mouse ileal myocytes

Activation of M_2 and M_3 receptors, which are co-expressed in all visceral smooth muscles including the gastrointestinal tract, with the stable muscarinic agonist carbachol provides the most physiologically relevant model for the study of isoflurane action on mI_{CAT} since all the above described signalling pathways linking muscarinic receptor activation to the openings of muscarinic receptor cation channels are fully operational. The latter are composed predominantly of TRPC4 (mediating about 85% of the response) and TRPC6 (remaining current) channels (8). In these experiments, 1 mM GTP was added to the pipette solution in

order to reduce mI_{CAT} desensitisation to a minimum. Under these conditions, mI_{CAT} at -40 mV declines only by about 15% over 3 min (23). Also, the decline occurs mainly due to a decrease in maximal conductance (i.e. the number of active channels), while positive shift of the activation curve is almost completely prevented (23).

Application of isoflurane (0.5 mM, as used to produce clinical anesthesia) at the peak response to carbachol caused a slow, but significant in size – by $63 \pm 11\%$ ($n=5$) at -40 mV, mI_{CAT} inhibition (Fig. 1A). The inhibitory effect was at least partially reversible after drug wash-out, as current amplitude reached a level comparable with normal desensitization process during such prolonged receptor agonist action (23). Kinetics of isoflurane inhibition of mI_{CAT} was very slow (half-maximal inhibition achieved within 1.1 ± 0.2 min; $n=5$), suggesting that any direct channel inhibition (e.g. ion pore occlusion) was not involved. Moreover, the characteristic steady-state I-V relationship of mI_{CAT} inhibited by isoflurane became more U-shaped at negative potentials (Fig. 1B), implying a positive shift of the activation curve as seen with decreased receptor occupancy by the agonist or reduced level of activation of G-proteins, for example induced by flash photolysis-released intracellular GDP β S (24, 25). Quantitative analysis of this phenomena involves construction of the activation curves (G-V relationships) and fitting them by the Boltzmann relations. These are shown in Fig. 1C. Thus, isoflurane not only depressed the maximal conductance (G_{max}), but also shifted the potential of half-maximal activation ($V_{1/2}$) positively: by 20.4 ± 5.4 mV ($n=4$) from an average $V_{1/2} = -85.0 \pm 4.5$ mV in control to an average $V_{1/2} = -64.7 \pm 3.0$ mV in the presence of isoflurane.

Since no significant shift of the activation curve normally occurs during receptor desensitisation with 1 mM GTP in the pipette solution (Zholos and Bolton, 1996), these observations strongly suggest that isoflurane acts at either the muscarinic receptor or G-protein sites, or both. To distinguish between these possibilities, in the next series of experiments mI_{CAT} was induced in a receptor-independent manner by GTP γ S infusion, which directly and irreversibly activates all available trimeric G-proteins.

3.2. Isoflurane inhibition of GTP γ S-induced mI_{CAT} shows several distinct features compared to carbachol-induced mI_{CAT}

Since isoflurane effect develops rather slowly, we first evaluated kinetics of the current response to intracellular infusion of GTP γ S (Fig. 2A). After break-through with 200 μ M of GTP γ S in the pipette solution, mI_{CAT} developed to reach a peak amplitude within 5–7 min (Fig. 2A, squares). The current response showed a characteristic double rectification in its I-V curves, which reversed close to 0 mV (Fig. 2B), while the change in its size over up to 20 min recording was minimal (Fig. 2A). At maximal response mI_{CAT} amplitudes of carbachol (50 μ M)- and GTP γ S (200 μ M)-induced currents were comparable: -227 ± 62 pA ($n = 5$) and -223 ± 32 pA ($n = 6$) at -40 mV, respectively. Corresponding maximal current densities were 9.0 ± 1.7 and 8.5 ± 1.1 pA/pF (not statistically significant difference, $P=0.82$).

Similarly to carbachol-induced mI_{CAT} , isoflurane significantly inhibited GTP γ S-induced current (Fig. 2A, circles), also producing a more U-shaped I-V curve (Fig. 2B). However, there were several distinct features of this inhibition compared to the inhibition of carbachol-induced currents. First, it developed more slowly, with half-maximal inhibition achieved

within 3.7 ± 0.7 min ($n=5$). Second, the extent of the inhibition was somewhat smaller, by $44 \pm 9\%$ ($n=6$) ($P < 0.05$ compared to carbachol-induced mI_{CAT}). Third, there was no or very little recovery of the response after isoflurane wash-out. Similarly to carbachol-induced mI_{CAT} isoflurane reduced G_{max} and shifted $V_{1/2}$ positively (Fig. 2C), but compared to carbachol the shift was less pronounced: by 13.0 ± 1.4 mV ($n=4$) from an average $V_{1/2} = -67.0 \pm 4.1$ mV in control to an average $V_{1/2} = -54.0 \pm 5.5$ mV in the presence of isoflurane. No such shift of $V_{1/2}$ occurred during 10 min recording in control (-68.9 ± 4.3 mV vs -67.5 ± 4.1 mV, $n=5$).

Taken together, the results suggest that disruption of the G-protein link by isoflurane is the predominant mechanism of its action. Isoflurane thus modifies channel gating (e.g. by causing $V_{1/2}$ shift) rather than causes direct muscarinic cation channel blockade. To further substantiate this point, we also analysed mI_{CAT} gating kinetics by fitting single exponential function to current voltage-dependent decline during voltage step to -120 mV, which occurs in keeping with its Boltzmann-type activation curve (Figs. 1C and 2C). Significant acceleration of mI_{CAT} deactivation by isoflurane was observed as a direct evidence of reduced mean open time of the channel in the presence of isoflurane (Fig. 2D). Similar effect was observed when G-proteins were inhibited by intracellular flash photolysis-released GDP β S from its “caged” precursor (24). Summary data and their statistical analysis further illustrating the difference in isoflurane potency to inhibit carbachol- and GTP γ S-induced currents are presented in Fig. 3.

3.3. Isoflurane inhibits agonist-induced intestinal contractions

To study the effect of isoflurane on intestinal smooth muscle contraction we used intestinal segments of mouse ileum or colon precontracted with carbachol ($50 \mu\text{M}$). Isoflurane (3 mM) was applied to the bath solution after carbachol-induced contraction reached plateau. As shown in Fig. 4A,B bath application of isoflurane significantly inhibited carbachol-induced contractile responses in both colon (12.26 ± 2.38 mN for control vs 8.14 ± 1.36 mN for isoflurane, $P < 0.01$) and ileum (14.52 ± 0.40 mN for control vs 11.18 ± 0.95 mN for isoflurane, $p < 0.05$). The inhibitory effect is consistent with the above described results on mI_{CAT} inhibition by isoflurane.

4. Discussion

General volatile anesthesia was introduced around 150 years ago. A variety of inhalation anesthetics has been developed since then, and the following are the most commonly used ones: isoflurane, sevoflurane, desflurane, halothane and enflurane. The mechanisms of general anesthetics action on cells remain largely unknown but at least two main molecular targets are being considered. Inhaled anesthetics diffuse rapidly from the alveoli into capillary blood. The first possible interacted structure is the lipid bilayer. Cell membrane is a high-ordered structure consisted of two phospholipids layers with closely packed hydrophilic heads outside and their hydrophobic tails inside. It is a well-organized and stable system, but temperature increase or interaction with small hydrophobic molecules like anesthetics can convert it into the liquid-state thus altering the membrane properties and functions (26). The second and much better understood group of targeting points is

represented by plasma membrane receptors and ion channels, both voltage-gated (most notable examples are two-pore-domain potassium channels, calcium and sodium channels) (Patel et al., 2001; Perouansky et al. 2003) and ligand-gated (Zhou, 2012). In particular, over the past ten years considerable information has been accumulated on the action of isoflurane on the pentameric ligand-gated ion channels, such as the nicotinic acetylcholine receptors (27), serotonin receptors (5-HT₃), as well as anion channels such as the γ -aminobutyric acid class A receptors and the glycine receptor (28, 29).

Receptor-operated and sensory cation TRP channels are currently arising as yet another large and important class of ion channels sensitive to both intravenous and inhaled anesthetics at subclinical concentrations (30). Isoflurane, a halogenated ether that causes analgesia and myorelaxation and that is used for general inhalation anesthesia, activated TRPA1 channels in mouse DRG neurones, but not TRPM8 and TRPV1. Moreover, TRPA1-evoked neurogenic inflammation was greater in mice anesthetized by isoflurane than sevoflurane (30).

Canonical TRP (TRPC) channels have recently appeared as crucial players in the control of smooth muscle function (31, 32). TRPC proteins present a subfamily of seven members (TRPC1-7) within the wider TRP superfamily. They are highly expressed in vascular and visceral smooth muscle cells in various organs, such as the uterus, the airways and the gastrointestinal tract. Inhalational halothane and chloroform, and intravenous propofol have been shown to inhibit TRPC5 activated by carbachol, lysophosphatidylcholine and Gd³⁺, but not the TRPM2 channel (33).

In this study we investigated TRPC4/6-mediated mI_{CAT} in ileal myocytes (8) and the effects of isoflurane on these channels. In many visceral smooth muscles, muscarinic receptors of two predominant subtypes, M₂ and M₃, are differentially coupled to distinct G-proteins (G_{i/o} and G_{q/11}, respectively), and both are indispensable for mI_{CAT} activation. Acting in synergy, they induce robust depolarizing muscarinic cation current via the two well-known signaling pathways: M₃/G_{q/11}/PLC/InsP₃/Ca²⁺ release and M₂/G_{i/o}. In turn, mI_{CAT} causes smooth muscle excitation and Ca²⁺ influx via L-type Ca²⁺ channels for the contractile response (5–7). Although TRPC4/6 channels are clearly involved in neurogenic contractions of intestinal smooth muscles (see Fig. 4 in (8)) there is highly non-linear relationship between mI_{CAT} amplitude and extent of membrane depolarisation, and hence stronger inhibition of carbachol-induced mI_{CAT} (Fig. 3A,C) compared to the inhibition of carbachol-induced intestine contraction (Fig. 4) by isoflurane at the same concentration should not be surprising. Moreover, there may be other factors explaining this difference, such as diffusional limitations present in multicellular preparations.

To clarify the targeting point(s) for isoflurane action within this complex system we compared its action on mI_{CAT} activated either by carbachol or by GTP γ S. In the latter scenario, muscarinic receptors are bypassed by the virtue of receptor-independent activation of trimeric G-proteins by GTP γ S. Although it was shown that halothane and chloroform inhibited TRPC5 channels likely through the direct anesthetic action (Bahnsi, 2008), we consider it unlikely that the inhibition of TRPC4/6-mediated mI_{CAT} by isoflurane was due to a channel block. First, the inhibition and recovery both developed rather slowly, and such

slow kinetics is not typical for direct channel pore occlusion by a drug. Second, and more important, there have been several notable differences in the extent and the rate of current inhibition between the two modes of mI_{CAT} activation – starting with muscarinic receptors or bypassing them. Such differences are unlikely to occur if TRPC4/6 proteins were the sole site of action for isoflurane since in both cases these channel proteins are the final effector molecules. Nevertheless, further more detailed studies are needed to confirm this hypothesis, especially at the single-channel level which allows a more detailed mechanistic analysis of channel conductance and gating. Diverse types of ion channels present binding sites for general anesthetics (34) and it may be premature to exclude TRPC4, and especially TRPC6 which produces only about 15% of the integral mI_{CAT} , as an isoflurane target based on the present results.

In contrast, inhibition of muscarinic receptors, G-proteins or their coupling by isoflurane seems a much more likely underlying mechanism of mI_{CAT} suppression. This is not only consistent with several previous studies showing that isoflurane can inhibit muscarinic receptors (14, 15) and disrupt muscarinic receptor - G protein coupling (16-18), but also has direct experimental support. Indeed, mI_{CAT} was not simply “scaled” down by isoflurane, as would be expected in case of simple channel block, but instead its biophysical properties were altered - specifically, the potential of half-maximal activation shifted positively while voltage-dependent deactivation kinetics was accelerated (Fig. 1 ; Fig. 2). We have previously extensively analysed these phenomena and found that activated G-proteins not only open muscarinic cation channels, but also control the voltage range of their activation and current kinetics (23, 25). Overall, the effect of isoflurane is equivalent to reducing agonist concentration or to inhibition of G-proteins by GDP β S. Thus, we can conclude that both muscarinic receptors and G-proteins (or their coupling) are affected by isoflurane, and this explains inhibition of both mI_{CAT} and contractile responses of intestinal smooth muscle to muscarinic agonists.

Considering possible species-dependent differences in the action of volatile anaesthetics on cholinergic excitation and contraction of intestinal smooth muscles it is important to note that, first, mI_{CAT} remains best-studied current in guinea-pig and mouse ileum, but remains to be studied in larger mammalian species. Some aspects of volatile anesthetics action have also been tested in other animal models, such as cats and horses, where it was shown that anesthetics can affect the function of many different ion channels and receptors, but rodents represent one of the most widely used model for the study of molecular and integrative physiological effects of isoflurane anesthesia (35).

In conclusion, the present results clearly show inhibitory action of clinically dosed isoflurane on TRPC4/6-mediated mI_{CAT} activated by carbachol or GTP γ S, but the intricate molecular and single-channel mechanisms of this effect remain to be studied further. Furthermore, in future work it would be very interesting to examine the action of other widely used inhalation anesthetics, such as desflurane, sevoflurane, halothane, enflurane, on TRPC4 as well as other types of receptor-operated TRPC channels.

5. Conclusions

The present study initiated investigation of molecular components affected by general anaesthesia in relation to the impaired gastrointestinal smooth muscle function, with focus on the muscarinic cation current predominantly mediated by TRPC4 channels activated in synergy by M₂ and M₃ receptors. The results so far show clear inhibition of mI_{CAT} by isoflurane at clinically relevant concentrations, with several notable differences between current responses initiated with or without muscarinic receptor involvement. The main targets of isoflurane appear to be muscarinic receptors, G proteins, or their coupling, while any direct muscarinic cation channel inhibition remains to be clarified. According to our results volatile anaesthetics inhibit agonist-induced intestinal smooth muscle contractions via suppression of the depolarising muscarinic cation current.

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Fig. 1.

Cationic current responses to carbachol and their inhibition by isoflurane. (A) Representative time course of 50 μ M carbachol-evoked mICAT and its inhibition by isoflurane (0.5 mM). Current amplitude at the holding potential of -40 mV was measured at 30 s interval. (B) Steady-state I-V relationships of mICAT at time points denoted a-c in panel A: a – at peak response to carbachol, b – during maximal current inhibition by isoflurane, and c – following drug wash-out. (C) Cation conductance activation curves (grey lines) fitted by the Boltzmann relation (smooth black lines) at the corresponding points, as indicated. A parallel shift of the activation curves (best-fit shared slope factor 15.6 mV) was observed – from $V_{1/2}$ of -91.0 mV (a) to $V_{1/2}$ of -57.8 mV (b) and to $V_{1/2}$ of -67.2 mV (c) as shows by the vertical dotted lines.

Fig. 2.
Cationic current responses to intracellular GTP γ S and their inhibition by isoflurane. (A) Normalized average time course of GTP γ S (200 μ M)-induced mI_{CAT} recorded at the holding potential of -40 mV in control (squares, $n=5$) and during isoflurane application (circles, $n=5$). (B) Superimposed steady-state I-V relationships of mI_{CAT} at corresponding time points *a* and *b* denoted in panel A. (C) Cation conductance activation curves corresponding to the I-V relationships shown in panel B fitted by the Boltzmann relation (smooth black lines) with $V_{1/2}$ values indicated by the vertical dotted lines: $V_{1/2}$ of -65.2 mV in control (*a*) and -52.6 mV in isoflurane (*b*). (D) mI_{CAT} voltage-dependent deactivation during voltage step from -40 to -120 mV at the same time points *a* and *b* fitted by single exponential function (smooth black lines).

Fig. 3.

Summary of the effects of isoflurane (0.5 mM) on mICAT evoked by 50 μ M carbachol (n=8) (A) or by 200 μ M GTP γ S (n=5) (B). In each cell, steady-state I-V relationships were measured at peak response using slow 6-s duration voltage ramps from 80 to -120 mV in control (circles) and at maximal inhibition by isoflurane (squares). Current amplitude was normalized by cell membrane capacitance to calculate current density. Normalised current density at -60 mV showed statistically significant differences, as indicated (C).

Fig. 4.

Inhibitory effect of isoflurane on carbachol-induced intestine contraction. Representative traces illustrating isometric contractions of ileum (A) or colon (B) segments in response to carbachol (50 μ M) followed by isoflurane application (3 mM) in the continuous presence of carbachol. (C) Summary of the measurements showing significant inhibition of carbachol-induced smooth muscle contraction by isoflurane. Maximal tension level was compared between carbachol and isoflurane (n=8).