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Sensitization of the Cardiac Na Channel to α_1-Adrenergic Stimulation by Inhalation Anesthetics

Evidence for Distinct Modulatory Pathways

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Background: **α_1-Adrenergic receptor stimulation has been shown to inhibit cardiac Na^+ current (I_{Na}). Furthermore, some form of synergistic interaction of α_1-adrenergic effects on I_{Na} in combination with volatile anesthetics has been reported. In this study, the authors investigated the possible role of G proteins and protein kinase C in the effects of halothane and isoflurane in the absence and presence of α_1-adrenergic stimulation on the cardiac I_{Na}.**

Methods: **The standard whole-cell configuration of the patch-clamp technique was used. I_{Na} was elicited by depolarizing test pulses from a holding potential of −80 mV in reduced Na^+ solution (10 mM). The experiments were conducted on ventricular myocytes enzymatically isolated from adult guinea pig hearts.**

Results: **The inhibitory effect of halothane (1.2 mM) and isoflurane (1 mM) on peak I_{Na} was significantly diminished in the presence of guanosine 5′-O-[2-thiodiphosphate (GDPβS). In myocytes pretreated with pertussis toxin (PTX), the potency of halothane was significantly enhanced, but the isoflurane effect was unchanged. In the presence of the protein kinase C (PKC) inhibitor bisindolylmaleimide (BIS), the effect of halothane was unchanged. In contrast, the effect of isoflurane on I_{Na} in the presence of BIS was significantly enhanced. The positive interaction between methoxamine and halothane was evident in the presence of G protein and PKC inhibitors. In contrast, the effect of methoxamine with isoflurane was additive in the presence of GDPβS or BIS.**

Conclusions: **Different second messenger systems are involved in the regulation of cardiac Na^+ current by volatile anesthetics. The effect of halothane involves a complex interaction with G proteins but is independent of regulation by PKC. In contrast, PKC is involved in the modulation of cardiac I_{Na} by isoflurane. In addition, non–PTX-sensitive G proteins may contribute to the effects of isoflurane. The positive interaction between methoxamine and anesthetics are independent of G proteins and PKC for halothane. In the case of isoflurane, the positive interaction with methoxamine is coupled to PTX-insensitive G proteins and PKC. (Key words: Halothane; isoflurane; methoxamine; patch clamp; second messenger; ventricular guinea pig myocytes; whole-cell configuration.)**

AT the cellular level, stimulation of α_1-adrenergic receptors has been shown to modify the activity of several different cardiac ion channels,[1] including Na^+ channels.[2] In guinea pig ventricular myocytes, α_1-adrenergic receptor activation by methoxamine inhibits Na^+ current amplitude in a concentration- and voltage-dependent manner.[2] Anesthetic potentiation of α_1-adrenergic effects in the heart has been suggested to contribute to the genesis of halothane-epinephrine dysrhythmias by markedly slowing cardiac conduction.[3] This is thought to be a key component in facilitating dysrhythmias by reentry mechanisms.[4] The mechanisms by which anesthetics and α_1-adrenergic stimulation depress conduction may involve a reduction of the fast cardiac inward Na^+ current (I_{Na}). A recent study showed a potentiation of α_1-adrenergic depressant effects on cardiac Na^+ current in ventricular myocytes in the presence of the volatile anesthetics halothane and isoflurane.[2] This positive interaction of α_1-effects in combination with anesthetics may contribute to the generation of dysrhythmias, especially in the ischemic heart.

The regulation of the cardiac Na^+ current by volatile anesthetics may involve G-protein-dependent pathways.[5] Furthermore, inactivation of the inhibitory G protein (G_i) seems to be involved in the facilitation of catecholamine-induced dysrhythmias in the heart.[6] However, not all α_1-adrenergic responses in cardiac tissue are mediated by a G_i protein.[1] Furthermore, many responses to α_1-adrenergic receptor stimulation are

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linked to protein kinase C (PKC).[1,7] A potential role of PKC in modulating anesthetic effects was shown recently, in which halothane and enflurane inhibited PKC activity.[8] However, for volatile anesthetics and α_1-adrenergic stimulation, the role of G proteins and PKC within the signal transduction pathway regulating the cardiac Na^+ channel is unclear.

The objective of the present study was to investigate the subcellular mechanisms underlying the depression of peak I_{Na} by (1) the volatile anesthetics halothane and isoflurane and by (2) methoxamine (an α_1-adrenergic agonist) in combination with anesthetics. The possible role of G proteins linking α_1-adrenoceptor activation and anesthetic action to the modulation of cardiac I_{Na} was also evaluated. Further, we examined the possible role of PKC in mediating the methoxamine and anesthetic effects on the cardiac Na^+ current. The whole-cell patch-clamp technique was used to measure the effects of anesthetics and methoxamine on the fast inward Na^+ current in single ventricular myocytes obtained from guinea pig hearts.

Methods

Unless stated otherwise, the experiments in this study were conducted under conditions described in an earlier article.[9] Briefly, single cardiac myocytes were obtained by retrograde perfusion of guinea pig hearts with an enzyme. Na^+ current was measured using the whole-cell configuration of the patch-clamp method. In most cases, linear leak current was digitally subtracted using the P/N method.[10] To exclude possible β-adrenergic activation, 100 nM propranolol was added to the external solution. Stock solutions of 10 mM methoxamine (Sigma Chemical Co., St. Louis, MO) and 1 mM propranolol (Sigma Chemical) were freshly prepared each day and diluted in the external bath solution. Pertussis toxin (List Biological Laboratories, Campbell, CA) was first prepared in distilled water. The final concentration of pertussis toxin (PTX) in Tyrode solution was 2 μg/ml. Bisindolylmaleimide (BIS; Calbiochem, La Jolla, CA) was initially prepared in dimethyl sulfoxide (Sigma Chemical) and further diluted in external solution. The desired final BIS concentration of 200 nM was achieved by 1:1000 dilution with the appropriate external solution. Dimethyl sulfoxide (0.1%) alone had no significant effect on I_{Na} ($n = 4$ cells).

Statistical analysis within one experimental group was computed using one-way repeated measures analysis of variance. Differences between treatment means were evaluated with the Bonferroni test. However, in some cases the tests for normality and equality of variance within groups were not satisfied. For those cases a one-way repeated measure analysis of variance on ranks (*post hoc* Student-Newmann-Keuls test) was used. When different groups of anesthetics were compared, data were expressed as percentage change and a two-way repeated measures analysis of variance was performed. Differences between group means were evaluated using the Bonferroni test. Statistical analysis was determined using commercially available software (SigmaStat, Jandel Scientific, Corte Madera, CA, and SuperANOVA, Abacus Concepts, Berkeley, CA). For experiments comparing shifts in steady-state inactivation, the predicted background shift was subtracted from the obtained shifts, as has been previously described,[11] before performing statistical analysis. A test was considered to be significant when $P < 0.05$. Data are presented as means ± SEM.

Results

Figure 1A shows halothane (1.2 mM) inhibition of peak I_{Na} by 38.5 ± 2.9%. Halothane inhibition of cardiac I_{Na} *via* a G-protein-dependent pathway is demonstrated in experiments using guanosine 5′-O-(2-thiodiphosphate) (GDPβS) in the pipette solution and in cells pretreated for 2-5 h with PTX. A nonhydrolyzable GDP analog, GDPβS competitively inhibits G protein activation by GTP and GTP analogs.[12] Petussin toxin inhibits activity of G_i and G_o proteins.[13] As shown in figure 1A, the effect of halothane was significantly diminished with GDPβS, decreasing current amplitude by 23.4 ± 2.5%. This corresponds with the results of our previous study.[9] In contrast to experiments with GDPβS, for myocytes pretreated with PTX, the potency of halothane is significantly enhanced, inhibiting I_{Na} by 54.1 ± 2.7%. Experiments including GDPβS in PTX-pretreated cells resulted in an inhibition of I_{Na} by 23.6 ± 3.1%, which is virtually unchanged compared with halothane effect with GDPβS alone. The result from the GDPβS and PTX combination indicated no further inhibition of PTX-sensitive G protein activity. To investigate the role of PKC in the halothane effect on I_{Na}, 200 nM BIS, a highly specific PKC inhibitor,[14] was added to the extracellular solution. The BIS concentration used in our experiments is approximately 65 times greater than the K_i for inhibition of PKC activity.[14] In the presence of BIS, the

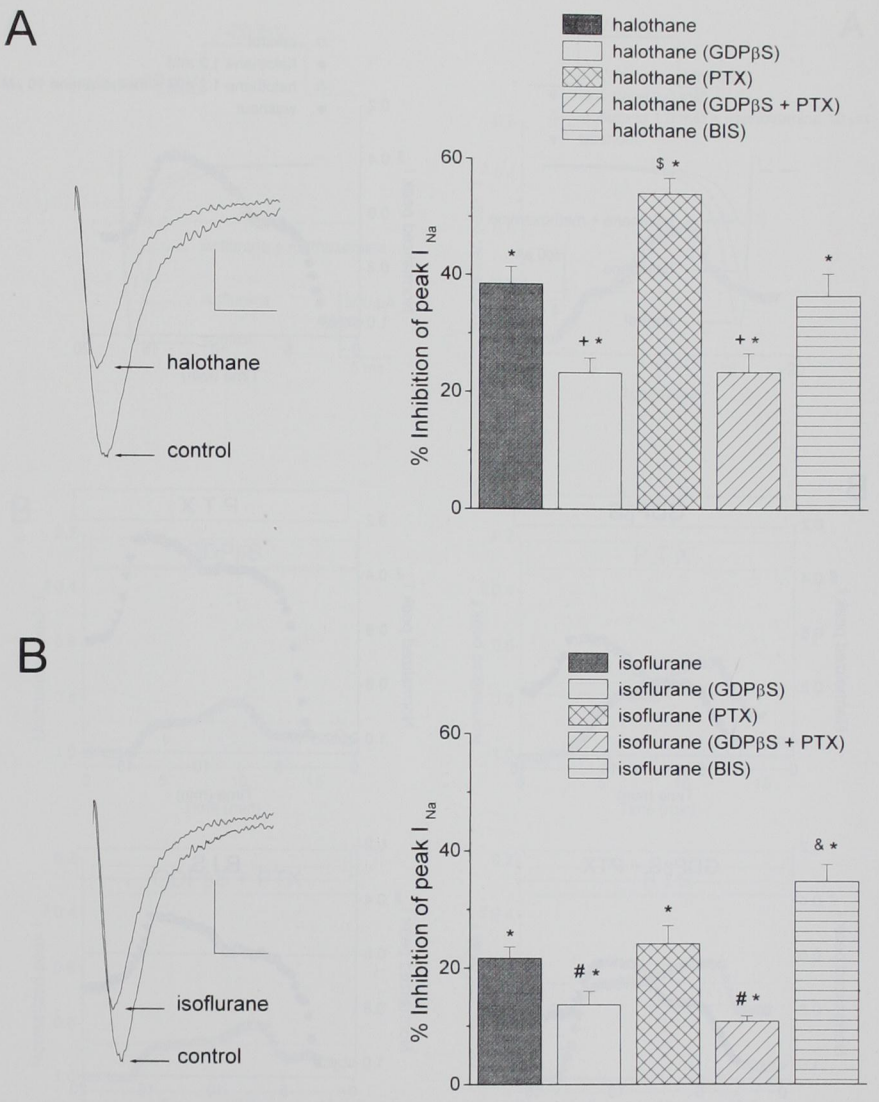

Fig. 1. Average depressant effects on peak Na^+ current by equianesthetic concentrations of 1.2 mM halothane (*A*) or 1 mM isoflurane (*B*). Sample peak Na^+ current traces (leak subtracted) are shown for control and after exposure to halothane and isoflurane. Calibration bars denote 500 pA and 5 ms. Data were recorded during a 50-ms test pulse to −20 mV from a V_H of −80 mV. Anesthetic effects are shown for untreated cells, in the presence of intracellular guanosine 5′-O-(2-thiodiphosphate) (GDPβS), after pretreatment of cells with pertussis toxin (PTX), in combination with GDPβS and PTX, and in the presence of bisindolylmaleimide (BIS). Averaged data of seven to nine cells per experimental group are shown. Error bars denote SEM; * significantly different than control; † significantly different than halothane; # significantly different than isoflurane.

depressant effect of halothane on Na^+ current amplitude remained unchanged (36.7 ± 4.2%) compared with the halothane effect alone (fig. 1A).

Figure 1B shows the average effects of isoflurane on I_{Na} in untreated myocytes and in combination with inhibitors. Isoflurane (1 mM) alone inhibited I_{Na} by 21.2 ± 2.0%. In the presence of GDPβS, the effect of isoflurane is significantly diminished, decreasing I_{Na} by 13.7 ± 2.4%. Unlike the effect of halothane, the effect of isoflurane on I_{Na} was not significantly different in PTX-pretreated cells compared with untreated cells, decreasing I_{Na} by 24.2 ± 3.1%. The combination of GDPβS and PTX with isoflurane depressed I_{Na} by 10.8 ± 0.9%, which was similar to the result obtained with GDPβS alone. Further contrasting the effect of halothane, the depressant action of isoflurane in the presence of BIS was significantly enhanced (35 ± 2.9%).

Figures 2A and 3A illustrate the effect of halothane and isoflurane, respectively, in combination with methoxamine on peak inward Na^+ current. For both anesthetics, the maximal suppressing effects were observed within 3 min after drug application. Methoxamine further decreased I_{Na} in the presence of either halothane or isoflurane. Washout of anesthetic and methoxamine, however, did not consistently result in complete recovery of the Na^+ current amplitude. As cited previously, this partial reversal can be attributed to the stabilization of the inactivated state

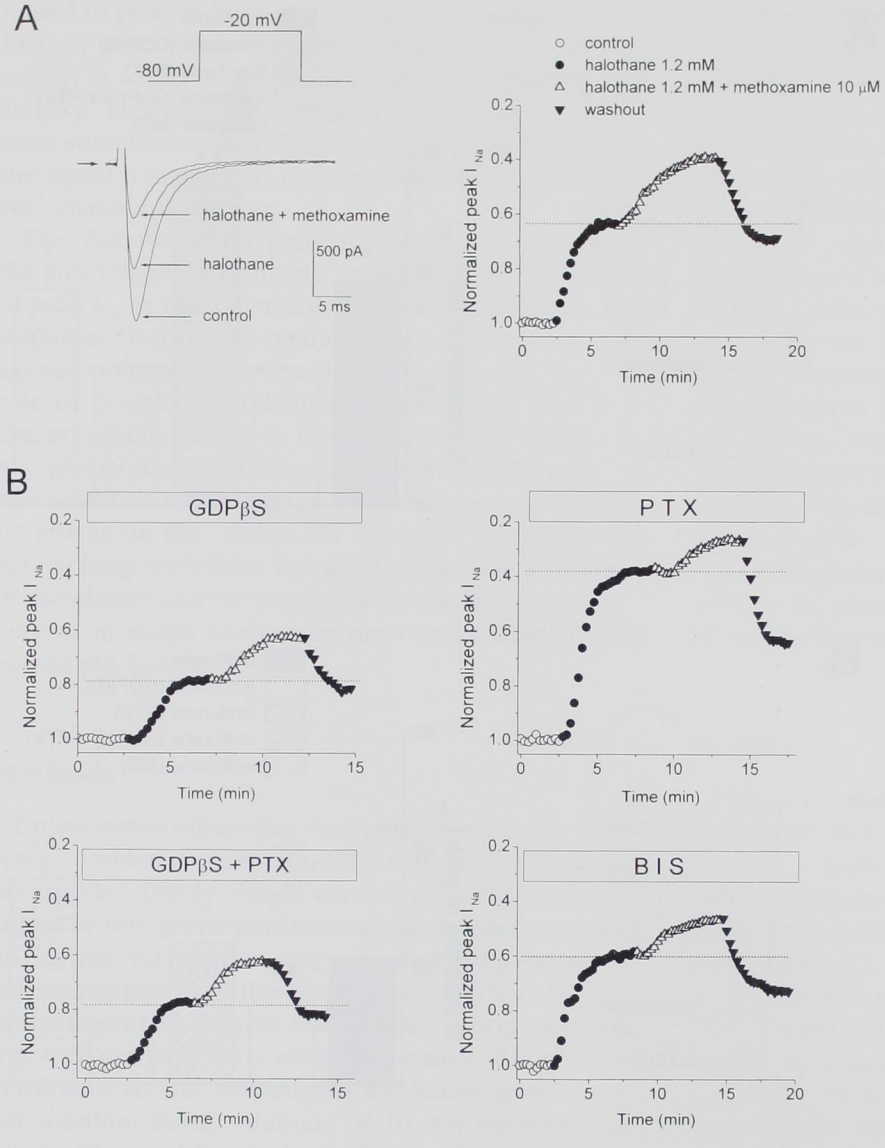

Fig. 2. Effects of halothane and methoxamine on peak Na^+ current during inhibition of G protein and protein kinase C (PKC). Data were obtained every 15 s from a 50-ms test pulse to 20 mV from a V_H of −80 mV. Time course of peak cardiac Na^+ current (I_{Na}) from five representative cells are shown in control (*open circles*), halothane exposure (*filled circles*), exposure to halothane plus methoxamine (*open triangles*), and washout (*filled triangles*). The additional reduction of I_{Na} by methoxamine was analyzed from the steady state obtained during anesthetic exposure, as shown by the dotted lines. (*A*) Peak Na^+ current traces and the corresponding time course are shown for an untreated cell. (*B*) Time course of peak I_{Na} during inhibition of G protein or PKC, as indicated. Guanosine 5′-O-(2-thiodiphosphate) (GDPβS; 20 mM) was added to the pipette solution. For pertussis toxin pretreatment (2 μg/ml), cells were preincubated for 2–5 h. Bisindolylmaleimide (200 nM) was added extracellularly.

of the channel by the anesthetics.[2,15] The effects of methoxamine in the presence of halothane or isoflurane were further investigated under conditions in which G protein and PKC activities were inhibited. The time courses of peak I_{Na} under the various conditions are depicted in figures 2B and 3B and the results are summarized in figure 4. To compare the additional reduction of I_{Na} by methoxamine in combination with anesthetics, data were analyzed from the steady state obtained during anesthetic exposure. Thus the current obtained after the maximal effect of anesthetic served as the new "control," as shown by the dotted lines in figures 2 and 3. The effect of methoxamine on I_{Na} in the presence of anesthetics was significantly enhanced compared with that of methoxamine alone (fig. 4A). The effect of methoxamine on I_{Na} in combination with halothane was also significantly enhanced under conditions of GDPβS, PTX, GDPβS plus PTX, and BIS (fig. 4B). In contrast to halothane, the effect of methoxamine with isoflurane appears to be additive in the presence of GDPβS, GDPβS plus PTX, or BIS (fig. 4B). However, a greater decrease of I_{Na} by methoxamine in combination with isoflurane was found in PTX-pretreated cells.

The effects of anesthetics and α_1-adrenergic stimu-

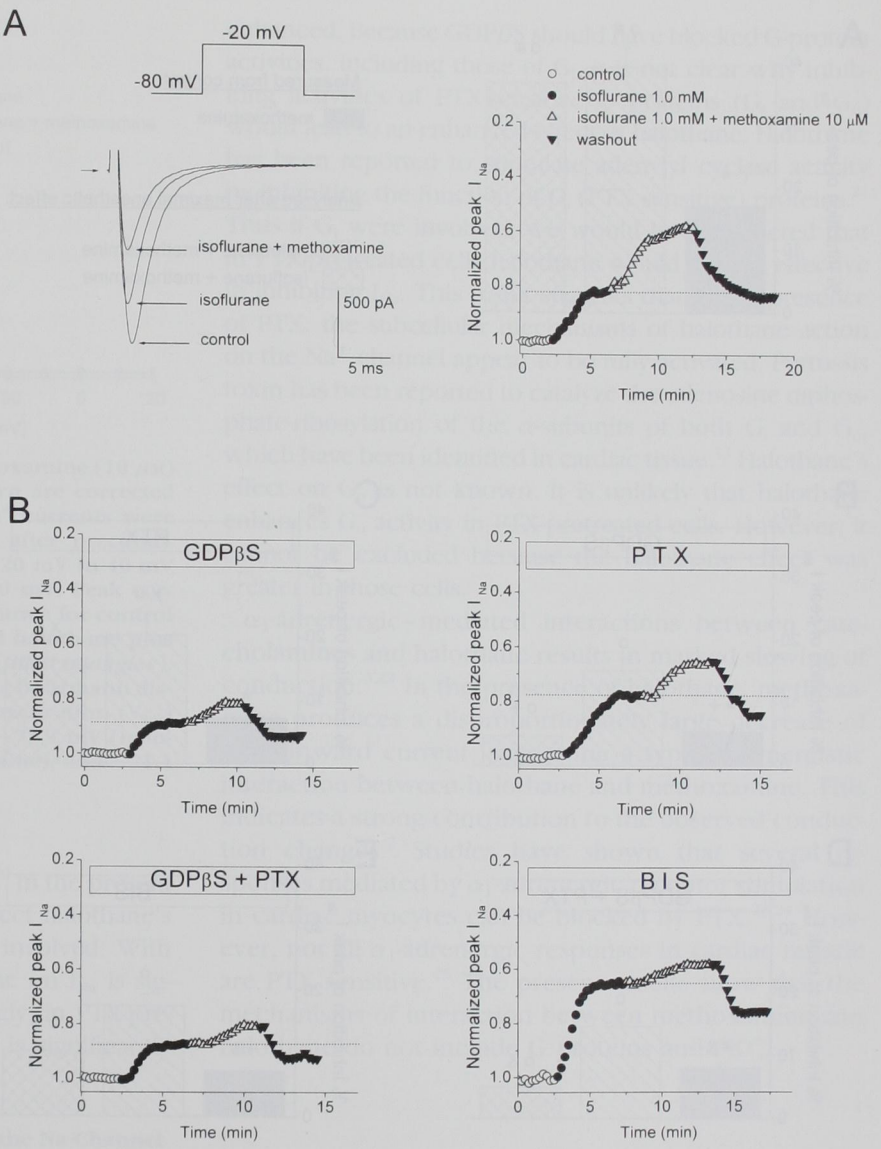

Fig. 3. Effects of isoflurane and methoxamine on peak Na^+ current during inhibition of G protein and protein kinase C (PKC). Data were obtained every 15 s from a 50-ms test pulse to 20 mV from a V_H of −80 mV. Time courses of peak cardiac Na^+ current (I_{Na}) from five representative cells are shown in control (*open circles*), halothane exposure (*filled circles*), exposure to isoflurane plus methoxamine (*open triangles*), and washout (*filled triangles*). The additional reduction of I_{Na} by methoxamine was analyzed from the steady state obtained during anesthetic exposure, as shown by the dotted lines. (*A*) Peak Na^+ current traces and the corresponding time course are shown for an untreated cell. (*B*) Time course of peak I_{Na} during inhibition of G protein or PKC, as indicated. Conditions are as described in figure 2.

lation by methoxamine on the steady-state inactivation parameters of the Na channel were also investigated. To distinguish drug-induced shifts from the spontaneous background shifts inherent for I_{Na},[16,17] steady-state inactivation curves were evaluated over time under control conditions. We have previously reported a rate of shift in steady-state inactivation of I_{Na} of -0.27 ± 0.01 mV/min under control (drug-free) conditions.[11] In the presence of either BIS or in PTX-pretreated cells, the rate of shift remained unchanged (n = 6, data not shown). However, GDPβS prevented the spontaneous shift in steady-state inactivation (n = 6 cells, data not shown). Steady-state inactivation was monitored after allowing for the diffusional exchange of GDPβS into the cell (approximately 25 min). Thus spontaneous shifts were corrected for, except in the presence of GDPβS. In the example shown in figure 5, which is corrected for the spontaneous shift, halothane alone decreased current amplitude at all potentials and shifted the potential for half-maximal inactivation ($V_{1/2}$) in the hyperpolarizing direction. Methoxamine in the continued presence of halothane further reduced Na^+ currents, and the steady-state inactivation curve was further shifted leftward. On washout of halothane and methoxamine, the current amplitude

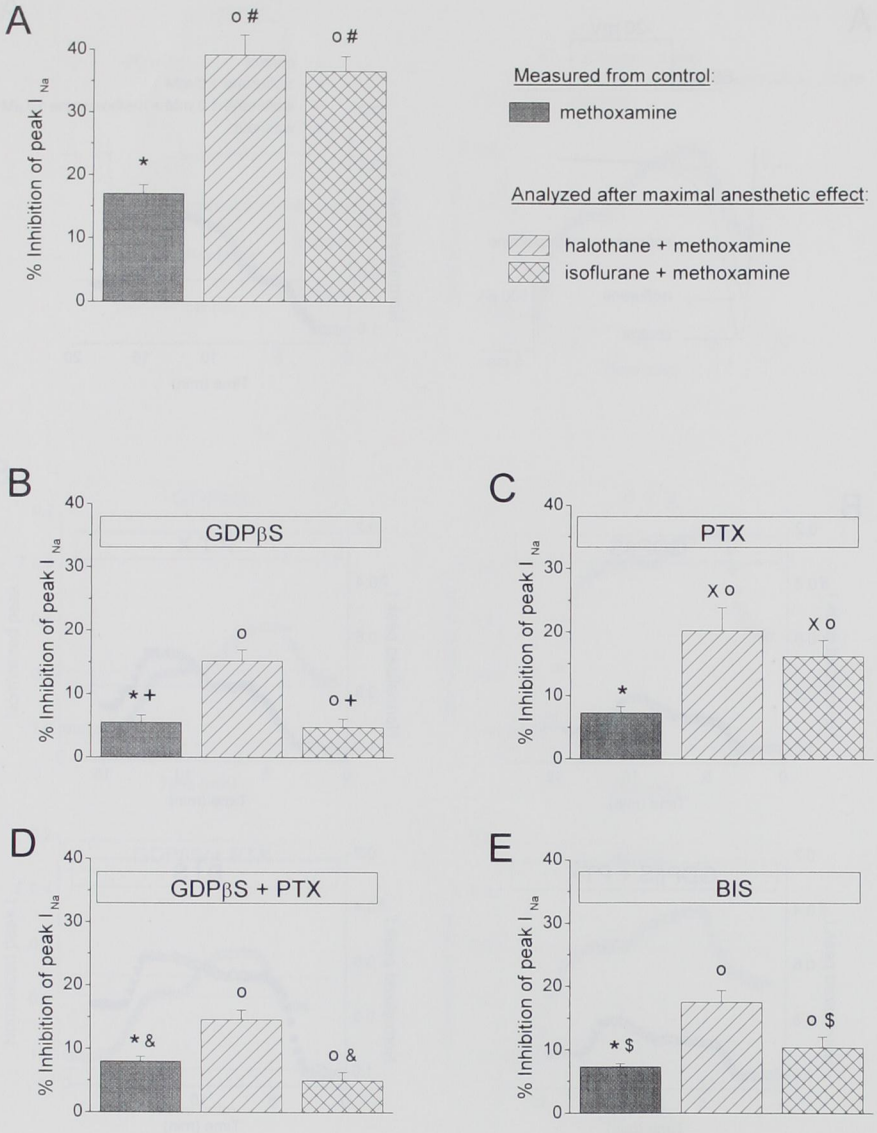

Fig. 4. Summary of the effects of methoxamine alone and in the presence of anesthetics, Guanosine 5′-O-(2-thiodiphosphate) (GDPβS), pertussis toxin (PTX), and bisindolylmaleimide (BIS) on peak cardiac Na^+ current (I_{Na}). In combination with halothane or isoflurane, the reduction of I_{Na} by methoxamine was measured after maximal anesthetic effect. The effects of methoxamine without anesthetics were measured from control. Data obtained from experiments are described in figures 2 and 3. (*A*) Average depressant effects for 10 μM methoxamine in the absence (17 ± 1.3%) and presence of either 1.2 mM halothane (39.1 ± 3.1%) or 1 mM isoflurane (36.5 ± 2.3%). (*B*) Inhibition of I_{Na} when GDPβS was added to the pipette solution: methoxamine (5.5 ± 1.1%), methoxamine in the presence of halothane (15.6 ± 1.7%), or isoflurane (5 ± 1.3%). (*C*) Depressant effects in PTX-pretreated cells: methoxamine (6.9 ± 1.1%), methoxamine in the presence of halothane (21.4 ± 3.7%), or isoflurane (15.4 ± 2.6%). (*D*) Inhibition of I_{Na} in combination with GDPβS and PTX: methoxamine (8 ± 0.8%), methoxamine in the presence of halothane (15 ± 1.5%), or isoflurane (4.9% ± 1.3%). (*E*) Inhibition of I_{Na} in the presence of BIS: methoxamine (7.3 ± 0.5%), methoxamine in the presence of halothane (18.4 ± 1.8%), or isoflurane (10.4 ± 1.7%). Each experimental group consists of 6 to 16 cells. Error bars denote SEM, * significantly different than control, ° significantly different from amplitude after anesthetic exposure, # significantly different than methoxamine, + significantly different than methoxamine plus halothane, × significantly different than methoxamine, & significantly different than methoxamine plus halothane, $ significantly different than methoxamine plus halothane.

at hyperpolarized potentials returned to control values. The halothane and methoxamine effects were readily reversible when using a holding potential of −110 mV. In all cases, no significant changes in the slope factor *k* were observed.

A summary of the effects of anesthetics and methoxamine on $V_{1/2}$ is shown in table 1. For halothane, the shift in steady-state inactivation was significantly enhanced in PTX-treated cells. For the isoflurane and methoxamine effects, there were no significant differences in shifts in $V_{1/2}$ in the presence of various inhibitors. No significant differences were found between methoxamine alone and methoxamine in combination with anesthetics in the presence or absence of the specific inhibitors.

Discussion

The regulation of the cardiac N channel by volatile anesthetics involves complex interactions of several distinct mechanisms. Studies have shown that volatile anesthetic action on the cardiac Na channel may, in part, be a result of direct interaction between the anesthetic and the channel protein.[18,19] In addition, anesthetic action on ion channel function may be induced by effects on the boundary

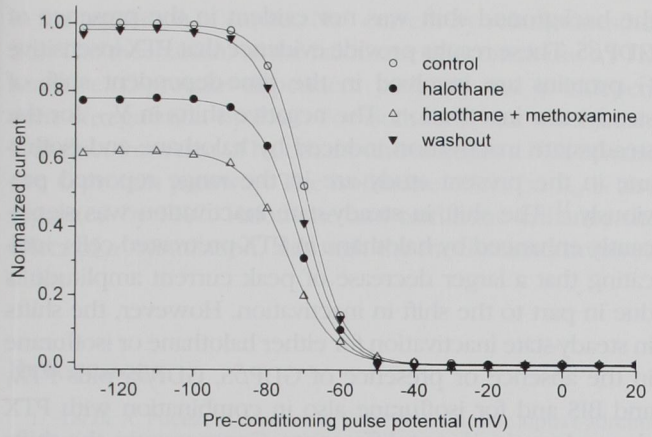

Fig. 5. Effect of halothane (1.2 mM) and methoxamine (10 μM) on steady-state inactivation. The shifts shown are corrected for spontaneous background shift. Peak Na^+ currents were recorded during test potential to −20 mV after preconditioning pulse potentials from −130 mV to +20 mV in 10-mV increments. The holding potential was −110 mV. Peak currents normalized to control conditions are shown for control (*open circles*), halothane (*filled circles*), and halothane plus methoxamine (*open triangles*) and washout (*filled triangles*). Steady-state inactivation curves were fitted to a Boltzmann distribution. The potentials for half-maximal inactivation ($V_{1/2}$) for this example were −69.5 mV (control), −72.9 mV (halothane), −74.2 mV (halothane plus methoxamine), and −71.4 mV (washout).

lipids surrounding the channel proteins.[18,20] In the present study, the PKC inhibitor, BIS, did not affect halothane's effect on I_{Na}, suggesting that PKC is not involved. With GDPβS, the depressant effect of halothane on I_{Na} is significantly diminished. However, surprisingly, in PTX-pretreated cells, the halothane effect on I_{Na} is significantly enhanced. Because GDPβS should have blocked G-protein activities, including those of G_i, it is not clear why inhibiting activities of PTX-sensitive G proteins (G_i and G_o) would lead to an enhanced effect of halothane. Halothane has been reported to stimulate adenylyl cyclase activity by inhibiting the function of G_i (PTX sensitive) proteins.[21] Thus if G_i were involved, we would have expected that in PTX-pretreated cells halothane would be less effective in inhibiting I_{Na}. This result suggests that in the presence of PTX, the subcellular mechanisms of halothane action on the Na^+ channel appear to be fully activated. Pertussis toxin has been reported to catalyze the adenosine diphosphate-ribosylation of the α-subunits of both G_i and G_o, which have been identified in cardiac tissue.[13] Halothane's effect on G_o is not known. It is unlikely that halothane enhances G_o activity in PTX-pretreated cells. However, it cannot be excluded because the halothane effect was greater in those cells.

α_1-adrenergic-mediated interactions between catecholamines and halothane results in marked slowing of conduction.[3,22] In the presence of halothane, methoxamine produces a disproportionately large decrease of peak inward current, suggesting a type of synergistic interaction between halothane and methoxamine. This indicates a strong contribution to the observed conduction changes.[2] Studies have shown that several responses mediated by α_1-adrenergic receptor stimulation in cardiac myocytes can be blocked by PTX.[23,24] However, not all α_1-adrenergic responses in cardiac muscle are PTX sensitive.[25] The present results show that the mechanisms of interaction between methoxamine and halothane do not include G proteins and PKC.

Table 1. Shifts in Steady-state Inactivation of the Na Channel

	Analyzed from Control			Analyzed after Maximal Anesthetic Effect	
Shift Δ $V_{1/2}$ (mV)	Halothane 1.2 mM	Isoflurane 1.0 mM	Methoxamine 10 μM	Methoxamine + Halothane	Methoxamine + Isoflurane
Untreated	−2.74 ± 0.24*†	−3.32 ± 0.22*	−1.60 ± 0.40*	−1.46 ± 0.37‡	−1.45 ± 0.42‡
GDPβS	−3.6 ± 0.40*†	−3.03 ± 0.58*	−0.85 ± 0.31*	−1.40 ± 0.25‡	−0.51 ± 0.52
PTX	−5.04 ± 0.36*	−3.60 ± 0.18*	−2.01 ± 0.39*	−2.13 ± 0.36‡	−1.15 ± 0.17‡
GDPβS + PTX	−3.69 ± 0.26*†	−2.67 ± 0.62*	−1.34 ± 0.53*	−1.14 ± 0.25‡	−0.55 ± 0.51
BIS	−3.44 ± 0.13*†	−4.17 ± 0.26*	−1.05 ± 0.43*	−0.86 ± 0.11‡	−0.92 ± 0.38

* Significantly different *vs.* control.

† Significantly different *vs.* halothane combined with PTX.

‡ Significantly different *vs.* anesthetic effect.

Changes in $V_{1/2}$ in the presence of halothane, isoflurane, and methoxamine with the indicated conditions are shown relative to control conditions. Changes in $V_{1/2}$ by methoxamine in the presence of anesthetics were determined after the maximal effect has occurred. Data are corrected for spontaneous background shifts (except in the presence of GDPβS) as described in Results section. Data shown are mean ± SEM. Average data for 6–7 cells in each experimental group are shown.

The mechanisms of action of isoflurane on I_{Na} appears to be distinct from those of halothane. The preceding study showed that isoflurane, like halothane, acts through a G-protein–dependent pathway but, unlike halothane, not *via* a cyclic adenosine monophosphate–dependent pathway.[2] Our result shows that the G-protein pathway involved in the isoflurane effect on I_{Na} does not involve PTX-sensitive G proteins. Thus this excludes the G_i and G_o proteins. This is supported by a study showing that isoflurane has no effect on either basal or stimulated adenylyl cyclase activity, which is regulated by the inhibitory G protein G_i.[26] Although we did not tested it in this study, one possible mechanism of action may be that isoflurane acts on cardiac Na channels through the direct membrane-delimited pathway *via* G_s because a regulation of cardiac I_{Na} by isoproterenol has been shown to include this pathway.[27] Another possibility includes a pathway *via* PTX-insensitive G proteins and PKC because stimulation of PKC has been shown to inhibit cardiac Na^+ current.[28] Our findings show a significant enhancement of the suppressing effect of isoflurane in the presence of the PKC inhibitor, BIS, indicative of an involvement of PKC. However, inhibition of PKC should have diminished the suppressing effect of isoflurane. Many studies have been done of the anesthetic effects on PKC. Enhancement of PKC-mediated smooth muscle vasoconstriction by isoflurane[29] and stimulation of brain PKC by halothane and propofol[30] have been reported. Inhibition of PKC by anesthetics in neuronal tissues have also been reported.[8] Attenuation of PKC by isoflurane in cardiac cells is unlikely because this would not lead to suppression of I_{Na}. Yet enhancement of PKC in cardiac ventricular myocytes would not explain the greater effect of isoflurane in the presence of BIS. If isoflurane suppressed I_{Na} *via* PKC stimulation, in the presence of BIS, the suppression would be less. Thus the mechanism for the greater isoflurane effect in the presence of BIS is unresolved.

Similar to halothane, the effect of methoxamine was significantly enhanced in combination with isoflurane compared with the methoxamine effect alone, indicating a type of synergistic interaction between these two agents. However, unlike the effect of halothane, the effect of isoflurane and methoxamine was additive in the presence of GDPβS, GDPβS plus PTX, and BIS. The isoflurane and methoxamine effect was unaffected in cells pretreated with PTX. Our findings clearly show that the interaction between isoflurane and methoxamine includes PTX-insensitive and PKC pathways.

The hyperpolarizing background shift, inherent in I_{Na} recordings under whole-cell configuration,[16] was unaffected by inhibition of PKC and in PTX-pretreated cells. However, the background shift was not evident in the presence of GDPβS. These results provide evidence that PTX-insensitive G proteins are involved in the time-dependent shift of steady-state inactivation. The negative shifts in $V_{1/2}$ for the steady-state inactivation induced by halothane and isoflurane in the present study are in the range reported previously.[11] The shift in steady-state inactivation was significantly enhanced by halothane in PTX-pretreated cells, indicating that a larger decrease of peak current amplitude is due in part to the shift in inactivation. However, the shifts in steady-state inactivation for either halothane or isoflurane in the absence or presence of GDPβS, GDPβS plus PTX, and BIS and for isoflurane also in combination with PTX showed no significant differences. Consequently, the shifts in steady-state inactivation in combination with inhibitors cannot account for the differential effects of each anesthetic on I_{Na}. Furthermore, inactivation shifts induced by methoxamine in the absence and presence of anesthetics and the different inhibitors were not significantly different. Thus the effects on I_{Na} by methoxamine and in combination with anesthetics cannot be explained by effects on steady-state inactivation.

Our results show a similar positive interaction between both anesthetics, halothane and isoflurane, with methoxamine. Thus α_1-stimulation alone cannot explain the observation that epinephrine or norepinephrine with halothane, more so than isoflurane, have synergistic negative dromotropic effects.[31,32] Our preceding study showed some form of synergistic interaction in suppressing I_{Na} only for halothane, and not for isoflurane, during β-adrenoceptor stimulation by isoproterenol.[9] Consequently, the less potent isoflurane effect in decreasing conduction velocity in combination with epinephrine appears to be related more to differential β-adrenergic–mediated effects rather than α_1-adrenergic effects. However, further experiments are necessary to examine the combined effects of volatile anesthetics and simultaneous α- and β-adrenoceptor stimulation, for example by epinephrine, on cardiac I_{Na}. In addition to the effect of α_1- and β-stimulation on I_{Na} being differentially affected by volatile anesthetics, the mechanisms of interaction are also different. As reported earlier, the enhanced effect of β-stimulation by halothane involves a G-protein–dependent, PKA-independent pathway.[9] In contrast, the interaction between α_1-stimulation and halothane is a G-protein–independent pathway and is also regulated by PKC. A type of synergistic interaction between α_1-stimulation and isoflurane includes a PTX-insensitive G-protein pathway and also PKC.

In summary, the present study provides strong evidence that intracellular signal transduction pathways are involved

in the regulation of cardiac Na^+ current by volatile anesthetics. The mechanism of the halothane effect includes a PTX-sensitive, G-protein–dependent pathway and is independent of regulation by PKC. For isoflurane, the mechanism is distinct from that of halothane and includes a PTX-insensitive G-protein pathway. The isoflurane effect on I_{Na} is also regulated by PKC. Halothane and isoflurane enhance the effect of α_1-stimulation, although the mechanisms involved differ.

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