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Modulation of the Cardiac Sodium Current by Inhalational Anesthetics in the Absence and Presence of β-Stimulation

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Background: **Cardiac dysrhythmias during inhalational anesthesia in association with catecholamines are well known, and halothane is more "sensitizing" than isoflurane. However, the underlying mechanisms of action of volatile anesthetics with or without catecholamines on cardiac Na channels are poorly understood. In this study, the authors investigated the effects of halothane and isoflurane in the absence and presence of β-stimulation (isoproterenol) on the cardiac Na^+ current (I_{Na}) in ventricular myocytes enzymatically isolated from adult guinea pig hearts.**

Methods: **A standard whole-cell patch-clamp technique was used. The I_{Na} was elicited by depolarizing test pulses from a holding potential of −80 mV in reduced Na^+ solution (10 mM).**

Results: **Isoproterenol alone depressed peak I_{Na} significantly by 14.6 ± 1.7% (means ± SEM). Halothane (1.2 mM) and isoflurane (1.0 mM) also depressed peak I_{Na} significantly by 42.1 ± 3.4% and 21.3 ± 1.9%, respectively. In the presence of halothane, the effect of isoproterenol (1 μM) was potentiated, further decreasing peak I_{Na} by 34.7 ± 4.1%. The halothane effect was less, although significant, in the presence of a G-protein inhibitor (GDPβS) or a specific protein kinase A inhibitor [PKI-(6-22)-amide], reducing peak I_{Na} by 24.2 ± 3.3% and 24 ± 2.4%, respectively. In combination with isoflurane, the effect of isoproterenol on I_{Na} inhibition was less pronounced, but significant, decreasing current by 12.6 ± 3.9%. GDPβS also reduced the inhibitory effect of isoflurane. In contrast, PKI-(6-22)-amide had no effect on isoflurane I_{Na} inhibition.**

Conclusions: **These results suggest two distinct pathways for volatile anesthetic modulation on the cardiac Na^+ current: (1) involvement of G proteins and a cyclic adenosine monophosphate (cAMP)–mediated pathway for halothane and, (2) a G-protein–dependent but cAMP–independent pathway for isoflurane. Furthermore, these studies show that the inhibition of cardiac I_{Na} by isoproterenol is enhanced in the presence of halothane, suggesting some form of synergistic interaction between halothane and isoproterenol. (Key words: Halothane; isoflurane; isoproterenol; patch clamp; second messenger; sodium current; ventricular guinea pig myocytes; whole-cell configuration.)**

CARDIAC dysrhythmias occur in approximately two thirds of patients undergoing anesthesia and surgery. Our present knowledge of how anesthetics and other drugs used during anesthesia contribute to the altered physiologic states responsible for dysrhythmias and other alterations in cardiac electrophysiology is far from complete.[1,2] Studies have shown that volatile anesthetics at clinically relevant concentrations directly depress the fast cardiac inward Na^+ current (I_{Na})[3,§] which may be responsible for slowing of impulse conduction and dysrhythmia generation due to abnormal conduction and reentry.[2,4] For example, halothane produces a modest depression of cardiac impulse conduction.[4-7]

The cellular electrophysiologic basis for the occurrence of cardiac dysrhythmias in association with endogenously released or exogenously administered catecholamines during inhalational anesthesia remains unclear.[8] Inhalational anesthetics differ substantially in their ability to potentiate the dysrhythmogenic actions of catecholamines, and halothane is more "sensitizing" than either isoflurane or enflurane.[2] Some investigators have found that β-adrenergic stimulation increases I_{Na} or the maximum rate of rise of the action potential upstroke (V_{max} in various cardiac preparations),[9,‖] whereas others have observed a decrease.[10-12] In cases in which the β-adrenergic agonist isoproterenol re-

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‖ Murray KT, Snyders DS, Bennet PB: Isoproterenol increases the cardiac sodium current in the presence of buffered Ca^{++}. Circulation 1990; 82(Suppl III): III-522A.

duced I_{Na}, the reduction was greatest when the fraction of channels in the inactivated state was increased.[11,12] Furthermore, it has been reported that the cardiac Na^+ channel is regulated by β-adrenergic receptors by both membrane delimited (direct) and cytoplasmic cyclic adenosine monophosphate (cAMP system) pathways *via* the signal transducing stimulatory G protein (G_s).[13]

Recently, halothane was reported to stimulate adenylylcyclase (AC) activity by inhibiting the function of the inhibitory G proteins (G_i).[14] In contrast, isoflurane and enflurane had no effect on either basal AC activity or forskolin-stimulated AC activity.[15] Thus the effects on both basal and stimulated AC activity may depend on the inhalational anesthetic.

The current study was designed to investigate the involvement of the cAMP second messenger system as one of the mechanisms by which the inhalational anesthetics halothane and isoflurane modulate cardiac I_{Na} in the absence and presence of β-adrenergic stimulation (isoproterenol). The whole-cell patch-clamp technique was used to measure directly the effects of these anesthetics and related drugs on the fast cardiac I_{Na}.

Methods

Cell Isolation

After approval by the institutional animal care and use committee, single ventricular cells were enzymatically isolated from adult guinea pigs weighing 200–300 g. The procedure of the cell isolation is the same as that described previously.[16] Briefly, single cardiac myocytes were obtained by retrograde perfusion of guinea pig hearts with an enzyme (collagenase; Gibco, Life Technologies, Grand Island, NY). Isolated cells were then transferred to a plexiglass chamber mounted on the stage of an inverted microscope (Olympus IMT-2, Tokyo, Japan). Only the rod-shaped cells with clear borders and striations were selected for these experiments and were used within 12 h of isolation.

Solutions

High-resistance seals and voltage clamp were attained in modified Tyrode solution containing 132 mM NaCl, 4.8 mM KCl, 1.2 mM $MgCl_2$, 10 mM HEPES, 5 mM dextrose, 1 mM $CaCl_2$, *p*H = 7.4 with NaOH. After establishment of a whole-cell voltage clamp, the external bath solution was changed to one that isolates the Na^+ channel current. This solution contained 115 mM CsCl, 10 mM NaCl, 10 mM HEPES, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5.5 mM glucose, and 3 mM CoCl, *p*H = 7.2 with CsOH. Cobalt and cesium were used as blockers for the L-type calcium channel and potassium channel currents, respectively. The standard pipette solution contained 11 mM EGTA, 1 mM $CaCl_2$, 10 mM HEPES, 2 mM Mg-ATP, 90 mM CsF, 60 mM CsCl, 10 mM NaF, *p*H = 7.3 with CsOH. In experiments in which G-protein activation was blocked, GDPβS (guanosine 5′-O-[2-thiodiphosphate], trilithium salt; 20 mM) was included in the pipette solution. In experiments in which protein kinase A activity was blocked, protein kinase inhibitor-(6-22)-amide (PKI, 10 μM) was added to the standard pipette solution. The PKI used in our experiments was heat stable with K of 1.7 nM.[17] To compare halothane and isoflurane at equianesthetic concentrations, agents were prepared in the following final bath concentrations: 1.2 mM halothane and 1 mM isoflurane. These concentrations at 22°C in Tyrode solution are equivalent to the following percentages in the gas phase (vol%)[18,19]: 1.96% for halothane and 2.60% for isoflurane. Anesthetic potencies are expressed as aqueous concentrations, which are relatively insensitive to temperature.[20] For the patch-clamp experiments, a system of glass syringes connected to a roller pump and a specially designed superfusion bath were used to allow a fast change of extracellular solution (<1 s). Glass syringes were used to ensure a constant inhalational anesthetic concentration superfused at 1.5 ml/min. Anesthetics were mixed by adding known aliquots of concentrated anesthetics to graduated syringes with extracellular solution. One milliliter of the superfusate was collected in a metal-capped 2-ml glass vial to prevent anesthetic losses. The superfusate concentrations of the inhalational anesthetics were determined by gas chromatography (head-space analysis using flame ionization detection Perkin-Elmer, Norwalk, CT; Sigma 3B gas chromatograph) as reported previously.[21] Stock solutions of isoproterenol-hydrochloride (1 mM) and forskolin (water-soluble, 7β-Deacetyl-7β-[Y-N-methylpiperazino]-butyryl, diHCl; 5 μM) were prepared every day and diluted in the external bath solution. GDPβS and forskolin were obtained from Calbiochem (La Jolla, CA), and isoproterenol-hydrochloride and PKI inhibitor peptide were purchased from Sigma Chemical Company (St. Louis, MO).

Recording Procedures and Data Analysis

Currents were recorded in the whole-cell configuration of the patch-clamp technique, as described by Hamill *et al.*[22] Due to the high density of Na^+ current in these ventricular cells, the following modifications

were made to prevent loss of voltage control: (1) small cells (60–80 pF) were selected to decrease membrane capacitance; (2) external Na^+ concentration was reduced to 10 mM to decrease the magnitude of Na^+ current (<2 nA); (3) pipette resistances ranged from 1.0 to 1.5 MΩ; (4) experiments were performed at room temperature (22°C); and (5) series resistance compensation was adjusted (to approximately 80%) to give the fastest possible capacity transient without feedback instability in the voltage clamp circuit. Under these conditions, the voltage error was <3 mV. Pipettes were pulled from borosilicate glass using a two-stage puller (Sachs-Flaming, PC-84, Sutter Instruments, Novato, CA) and heat polished (Narishige microforge, MF-83, Tokyo, Japan). Current was recorded by a List EPC-7 patch-clamp amplifier (Adams & List Associates, Great Neck, NY), and the output was lowpass filtered at 3 kHz to reduce high-frequency noise. All data were digitized and stored for later analysis with the pCLAMP software package (Axon Instruments, Foster City, CA).

After rupturing the membrane and achieving whole-cell voltage-clamp conditions, we allowed the cell to equilibrate for at least 15 min before acquiring data. When the internal perfusion of drugs was required, recordings were made after at least 25 min had elapsed to allow for diffusional exchange between the pipette and the cell. To determine peak I_{Na}, cells were held at a holding potential (V_H) of −80 mV, and whole cell currents were elicited by 30 ms test depolarizations from −80 mV to +30 mV in 10-mV increments. To monitor changes in the peak I_{Na}, 50-ms pulses were applied every 15 s from a holding potential of −80 mV to the test potential of −20 mV or −30 mV, the potential at which the peak I_{Na} occurred. Recordings were made from cells when the current amplitude during control was stable for at least 5 min. To test for effects of drugs (isoproterenol or forskolin) in combination with anesthetics, drugs were applied after maximal depressant effects of anesthetics had occurred. To compare the additional reduction of I_{Na} by these agents, data were analyzed from the steady-state during anesthetic exposure. Thus the current obtained after maximal effect of anesthetic served as the "new" control. To monitor steady-state inactivation of I_{Na}, cells were subjected to 500-ms preconditioning pulse potentials between −130 (the holding potential) and +20 mV and then stepped to the test pulse potential of −20 mV (24 ms), where peak Na^+ current occurred. Peak currents evoked by the test potential were normalized to the maximum current (I_{max}) obtained during the test potentials. Steady-state inactivation was fitted to a Boltzmann distribution[23] described by:

$$I/I_{max} = 1/\{1 + \exp[(V - V_{1/2})/k]\}$$

where V is the preconditioning potential, $V_{1/2}$ is the potential at which half maximum inactivation occurs, and k is the slope factor. Observed shifts in $V_{1/2}$ for the steady-state inactivation were corrected for spontaneous background shift over time as described previously.[16,24]

Data are expressed as means ± SEM. Statistical differences within one experimental group were determined from raw data using one-way repeated measures analysis of variance. Differences between treatment means were evaluated using 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 a 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 computed using commercially available software (SigmaStat, Jandel Scientific, Corte Madera, CA; SuperANOVA; Abacus Concepts, Berkley, CA). A test result was considered significant when $P < 0.05$.

Results

Figure 1 illustrates the effects of halothane or isoflurane and isoproterenol on the peak inward Na^+ current monitored over time. As shown by recordings from representative cells, I_{Na} was initially depressed by halothane (1.2 mM; fig. 1A) or isoflurane (1 mM; fig. 1B), respectively. As demonstrated, efficacy of I_{Na} block by isoflurane was less than that of halothane at approximately equianesthetic concentrations. For both halothane and isoflurane, the maximal effects were observed within 3 min after drug application. To determine the effect of anesthetic plus β-adrenergic stimulation on peak I_{Na}, isoproterenol (1 μM) was added to the superfusing solution in addition to anesthetic after the maximal anesthetic effects were achieved. To compare the additional reduction of I_{Na} by isoproterenol, 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

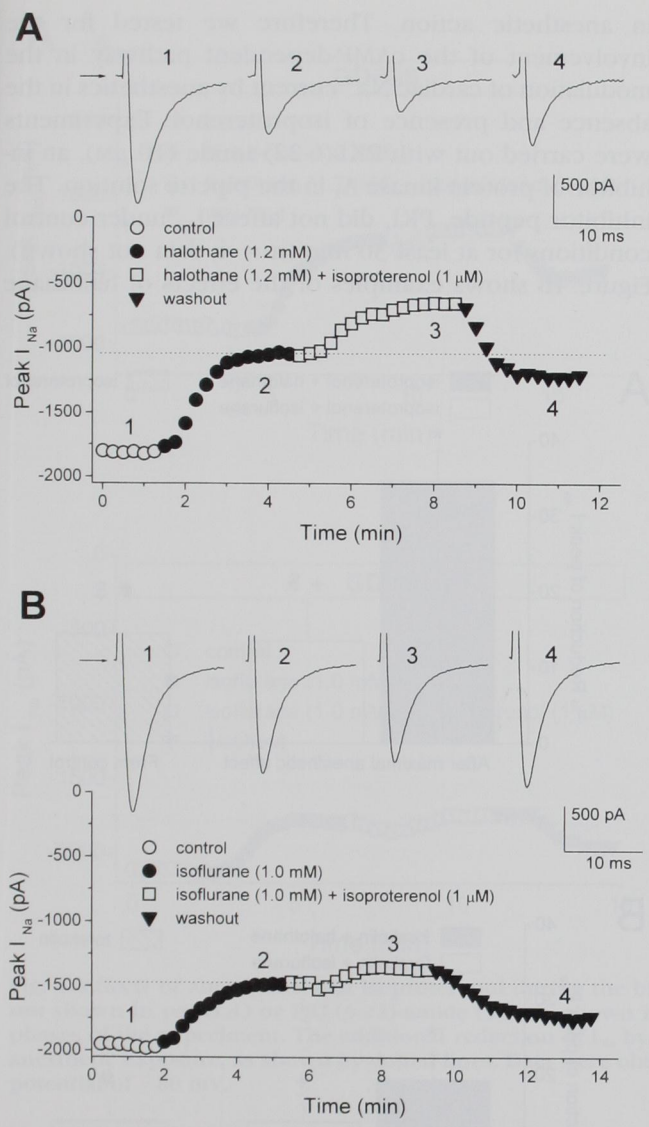

Fig. 1. Inhibition of whole-cell Na^+ current by anesthetics in combination with isoproterenol. The effects of equianesthetic concentrations of halothane (1.2 mM, panel *A*) and isoflurane (1.0 mM, panel *B*) on peak Na^+ current traces in the absence and presence of isoproterenol (1 μM) are shown. The current traces were recorded during a 30-ms test pulse to −20 mV from a holding potential of −80 mV. The corresponding time courses show peak I_{Na} amplitude in control (1), anesthetic (2), anesthetic plus isoproterenol (3), and washout periods (4). The additional reduction of I_{Na} by isoproterenol was analyzed from the steady-state obtained during anesthetic exposure, as shown by the dotted lines. During the experimental protocol, current was elicited every 15 s.

the dotted lines in figure 1. In the continued presence of anesthetics, isoproterenol further decreased peak I_{Na}. The additional reduction induced by isoproterenol was much more pronounced in combination with halothane than with isoflurane. A noticeable feature of the effects of both anesthetics on I_{Na} was that the effects were not completely reversible. This was not due to a "run down" of I_{Na}. Under control conditions, in the absence of drugs, I_{Na} was stable for at least 30 min (n = 6 cells, data not shown). This partial reversibility may be due to the anesthetics stabilizing the inactivated state of the channel.[25]

To test whether G-protein pathways are involved in the anesthetic effects during the β-adrenergic stimulation, we activated AC directly by forskolin, thus bypassing the G-protein pathway. The experimental protocol used was similar to that shown in figure 1. After the maximal effect of anesthetic on I_{Na} reached a steady state, forskolin (5 μM), in the continued presence of halothane or isoflurane, further decreased I_{Na} (fig. 2).

Figure 3 summarizes the effects of isoproterenol or forskolin either alone or combined with anesthetics on I_{Na}. Isoproterenol (1 μM) alone reduced peak I_{Na} in the absence of anesthetics by 14.6 ± 1.7% (fig. 3A). In combination with halothane, the depressant effect of isoproterenol was approximately three times greater, reducing I_{Na} by 34.7 ± 4.1%, indicating some form of synergistic interaction between halothane and isoproterenol. This reduction was measured after the anesthetic effect reached steady-state. In contrast, the Na^+ current was depressed by 12.6 ± 4.9% by isoproterenol in combination with isoflurane. The effects of forskolin in the absence or presence of either halothane or isoflurane were rather unchanged (no significant difference; fig. 3B). Forskolin alone decreased I_{Na} by 13.8 ± 2.4%. In contrast to the dramatic enhanced effect of isoproterenol in the presence of halothane, the effect of forskolin on I_{Na} was not altered in the presence of halothane. These findings suggest that a G-protein mechanism not mediated through AC may be involved in the synergistic interaction between halothane and isoproterenol. For isoflurane, no differences in the reduction of peak I_{Na} were observed with either isoproterenol or forskolin. Thus the effects of isoproterenol and forskolin, in combination with isoflurane, are not enhanced.

Further tests for the involvement of G proteins in the modulation of I_{Na} by anesthetics alone and during β-adrenergic stimulation were done with GDPβS in the intracellular (pipette) solution. GDPβS, a nonhydrolyzable GDP analog, competitively inhibits G-protein activation by GTP and GTP analogs.[26] Figure 4A shows in representative cells the time course of peak I_{Na} in the presence of GDPβS in the pipette during control exposure to anesthetics and isoproterenol in combination

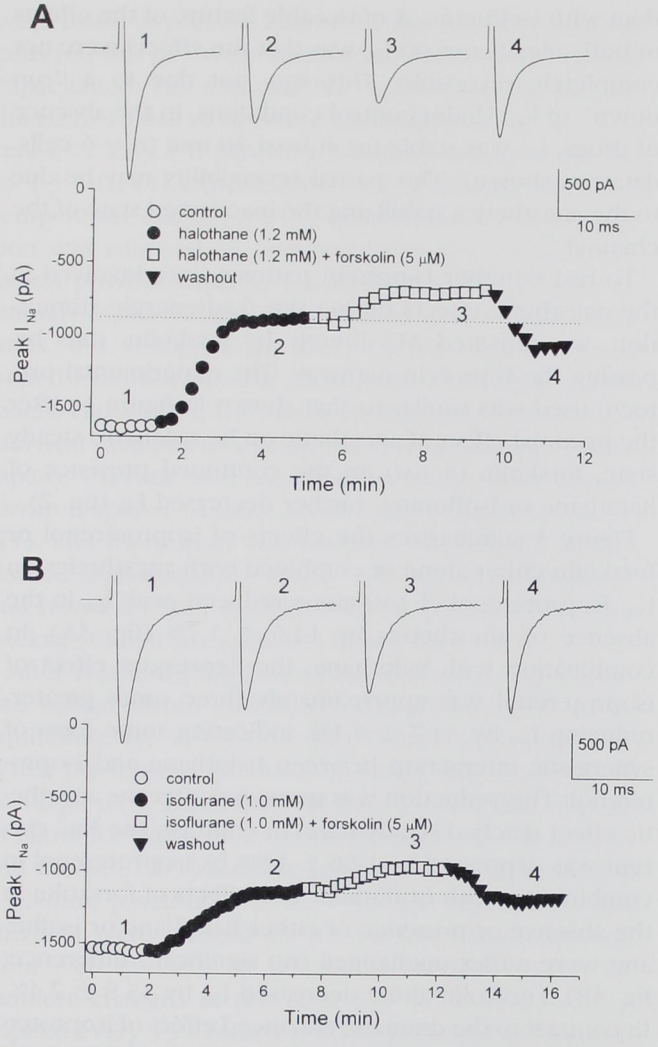

Fig. 2. Inhibition of Na^+ current by anesthetics in combination with forskolin. The effects of equianesthetic concentrations of halothane (1.2 mM, panel *A*) and isoflurane (1.0 mM, panel *B*) on peak Na^+ current traces in the absence and presence of forskolin (5 μM) are shown. Whole-cell voltage protocol was identical to that described in figure 1. The corresponding time courses show peak I_{Na} amplitude in control (1), anesthetic (2), anesthetic plus forskolin (3), and washout periods (4). The additional reduction of I_{Na} by forskolin was analyzed from the steady-state obtained during the anesthetic exposure, as shown by the dotted lines.

with halothane or isoflurane. In control experiments, GDPβS at 20 mM prevented I_{Na} inhibition by extracellular application of isoproterenol (n = 6, data not shown). GDPβS alone at this concentration had no effect on I_{Na} over 30 min (n = 4, data not shown).

β-adrenergic stimulation increases cytosolic cAMP. Thus phosphorylation of the channel protein by protein kinase A, as a result of increased cAMP, may be involved in anesthetic action. Therefore we tested for the involvement of the cAMP-dependent pathway in the modulation of cardiac Na^+ current by anesthetics in the absence and presence of isoproterenol. Experiments were carried out with PKI-(6-22)-amide (10 μM), an inhibitor of protein kinase A, in the pipette solution. The inhibitor peptide, PKI, did not affect I_{Na} under control conditions for at least 30 min (n = 4; data not shown). Figure 4B shows examples of the effects of halothane

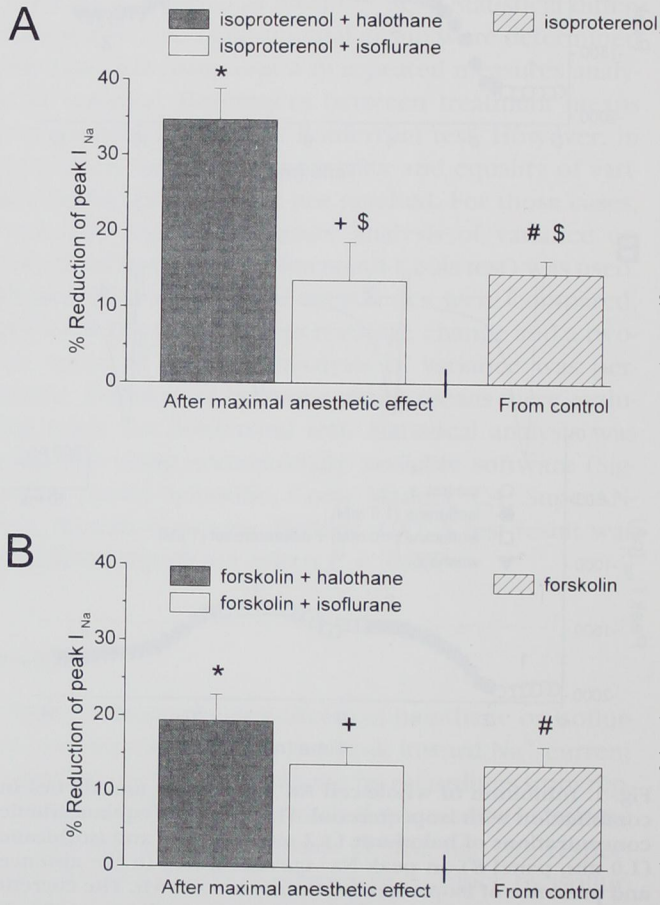

Fig. 3. Summary of the inhibition of Na^+ current by isoproterenol (1 μM, panel *A*) and forskolin (5 μM, panel *B*) alone and in the presence of anesthetics. In combination with halothane or isoflurane, the reduction of I_{Na} by either isoproterenol or forskolin was measured after maximal anesthetic effect. The effects on I_{Na} by isoproterenol and forskolin without anesthetics were measured from control (anesthetic free). Data were obtained from the experiments described in figures 1 and 2. Average values (means ± SEM) from 9 to 12 cells per experimental set are shown. * Significantly different from steady-state I_{Na} amplitude after halothane exposure; + significantly different from steady-state I_{Na} amplitude after isoflurane exposure; $ significantly different from isoproterenol in combination with halothane; # significantly different from control (anesthetic free). $P < 0.05$ was considered significant.

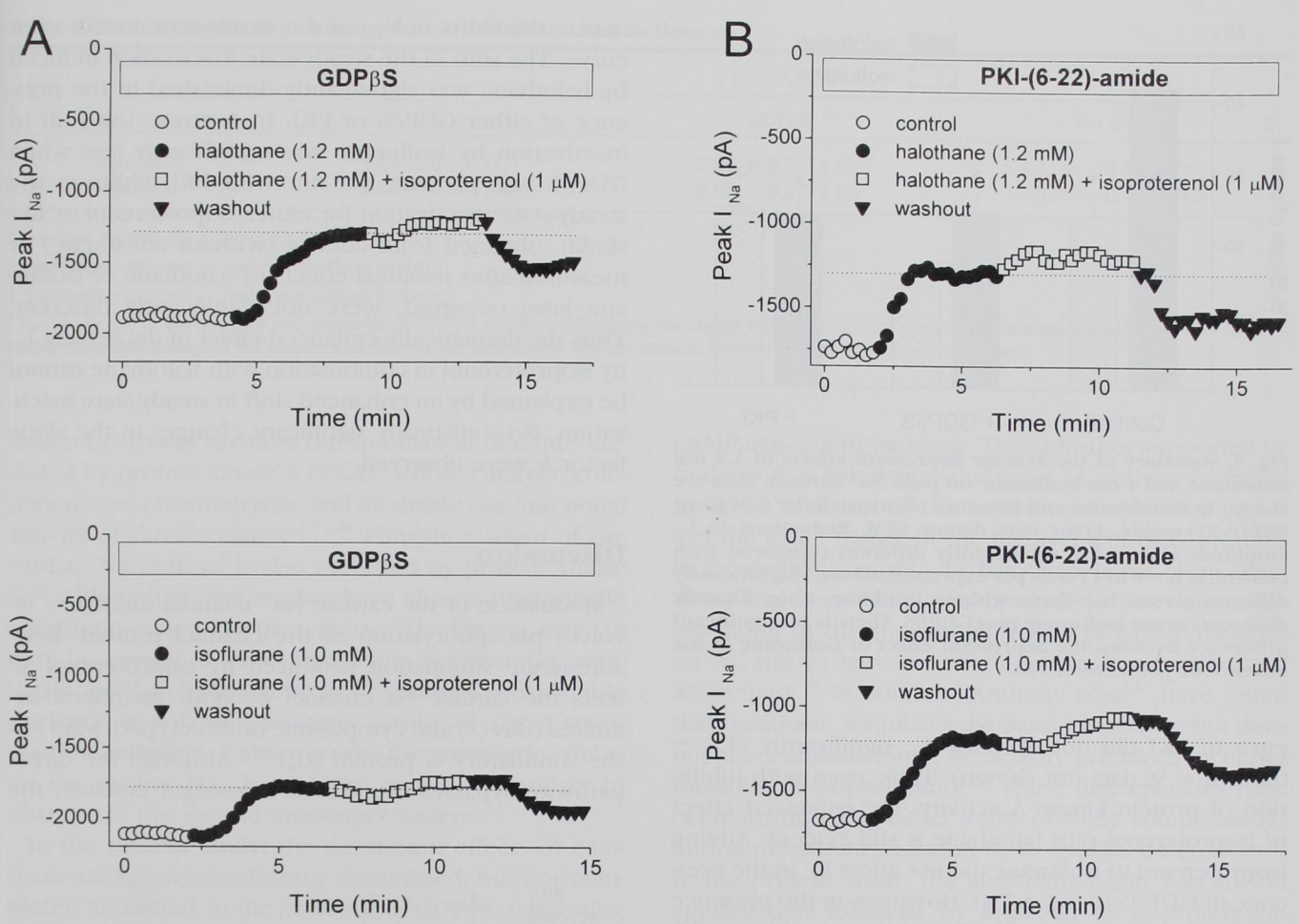

Fig. 4. Effects of anesthetics and isoproterenol during the block of G protein and protein kinase A. GDPβS (trilithium salt; 20 mM shown in panel *A*) or PKI-(6-22)-amide (10 μM, shown in panel *B*) were added to the standard pipette solution during all phases of the experiment. The additional reduction of I_{Na} by isoproterenol was analyzed from the steady state obtained during anesthetic exposure, as shown by dotted lines. Data were obtained every 15 s from a 30-ms test pulse to −20 mV from a holding potential of −80 mV.

or isoflurane and isoproterenol on peak I_{Na} in the presence of PKI.

Figure 5 summarizes the effects of halothane and isoflurane alone and in the presence of GDPβS or PKI. Halothane alone (1.2 mM) decreased I_{Na} by 42.1 ± 3.4%. With GDPβS in the pipette, the depressant effect of halothane was significantly diminished. When G-protein activity was blocked, halothane inhibited I_{Na} by 24.2 ± 3.3% compared with control. Similarly, in the presence of PKI, halothane decreased I_{Na} by 23.9 ± 2.4%. With isoflurane, the anesthetic alone decreased the peak I_{Na} by 24.1 ± 3.9%. When combined with GDPβS, the blocking effect of isoflurane was significantly diminished, decreasing I_{Na} by 15.0 ± 2.2%. However, unlike the effect of halothane, the effect of isoflurane on I_{Na} was similar in the absence and presence of PKI-(6-22)-amide. There was no significant difference in the sensitivity of I_{Na} to isoflurane with or without inhibition of protein kinase A activity.

Figure 6 summarizes the effects of isoproterenol on I_{Na} in combination with either halothane or isoflurane. Steady-state obtained during exposure to anesthetics alone served as a new "control" (shown by dotted lines in figures 1 and 4). In the presence of halothane, isoproterenol decreased peak I_{Na} by 34.7 ± 4.1%. With GDPβS or PKI-(6-22)-amide in the pipette solution, the effect of isoproterenol in combination with halothane was significantly but not completely attenuated, decreasing I_{Na} by 5.5 ± 3.3% and 10.1 ± 3.6%, respectively. Extracellular application of isoproterenol alone in the pres-

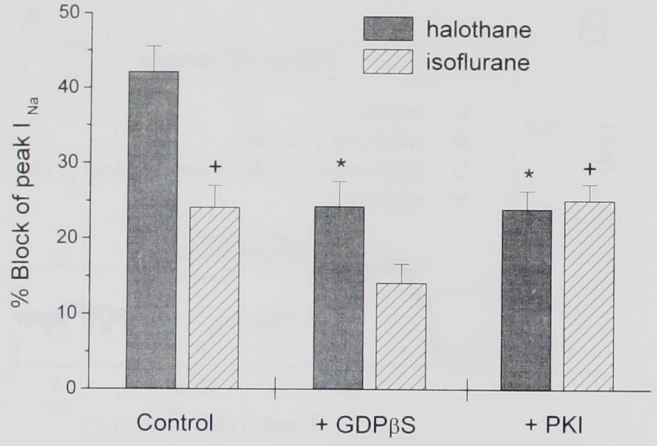

Fig. 5. Summary of the average depressant effects of 1.2 mM halothane and 1 mM isoflurane on peak Na^+ current. Data are shown in the absence and presence of intracellular GDPβS or PKI-(6-22)-amide. Error bars denote SEM. Reductions in I_{Na} amplitude shown are significantly different compared with control, at n = 6 to 12 cells per experimental set. * Significantly different *versus* halothane without inhibitor; + significantly different *versus* isoflurane plus GDPβS. There is no significant difference between the depressant effect of isoflurane in the absence or presence of PKI.

ence of PKI did not decrease I_{Na} significantly (1.9 ± 0.8%; n = 9; data not shown). Thus, even with inhibition of protein kinase A activity, the enhanced effect of isoproterenol plus halothane is still evident. Adding isoproterenol to isoflurane did not affect I_{Na} in the presence of GDPβS (0.9 ± 3.7%). However, in the presence of PKI, the depression of I_{Na} by isoproterenol in combination with isoflurane is still evident, decreasing current amplitude by 9.4 ± 3.1%.

We further investigated the effects of anesthetics and isoproterenol on the steady-state inactivation parameters of the Na channel. Spontaneous hyperpolarizing shifts in steady-state inactivation in the absence of drugs have been reported.[24,27] To distinguish drug-induced and spontaneous shifts, steady-state inactivation curves were evaluated over time under control conditions. We 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.[16] In the presence of intracellular PKI, the rate of shift remained unchanged (n = 6; data not shown). However, in the presence of GDPβS, no spontaneous shift in steady-state inactivation was observed in six cells. In both cases, steady-state inactivation was monitored after allowing for the diffusional exchange of PKI or GDPβS into the cell (approximately 25 min). Thus spontaneous shifts were corrected for except in the presence of GDPβS. Tables 1 and 2 summarize the shifts in $V_{1/2}$ of the steady-state inactivation curve. The shift in the steady-state inactivation induced by halothane was significantly diminished in the presence of either GDPβS or PKI. In contrast, the shift in inactivation by isoflurane was significantly less when GDPβS was present, but not with PKI. Shifts in the steady-state inactivation for either isoproterenol or forskolin, obtained from control (without anesthetic) or measured after maximal effects of halothane or isoflurane have occurred, were not significantly different. Thus the dramatically enhanced effect of decreasing I_{Na} by isoproterenol in combination with halothane cannot be explained by an enhanced shift in steady-state inactivation. No statistically significant changes in the slope factor *k* were observed.

Discussion

Modulation of the cardiac Na^+ channel intimately involves phosphorylation of the channel protein. Beta-adrenergic stimulation produced by isoproterenol affects the cardiac Na channel by both membrane-delimited (direct) and cytoplasmic (indirect) pathways *via* the stimulatory G protein (G_s).[9,13] Although the direct pathway bypasses the second-messenger cascade, the

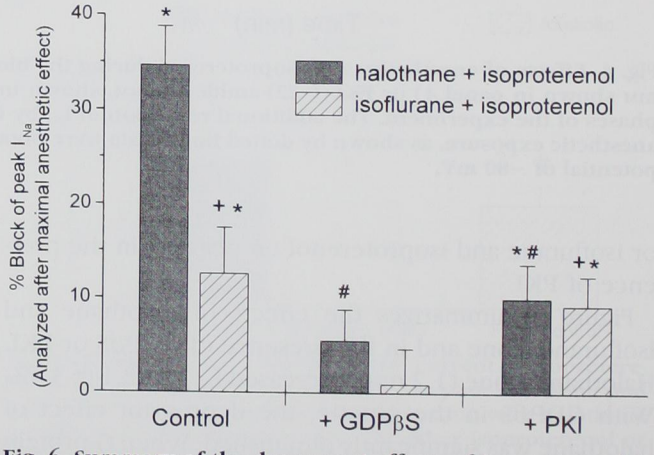

Fig. 6. Summary of the depressant effects of isoproterenol in combination with anesthetics. Data from 6 to 12 cells per experimental set are shown in the absence and presence of GDPβS or PKI-(6-22)-amide. The reduction of peak I_{Na} by isoproterenol was analyzed from the steady state obtained during anesthetic exposure, as shown by the dotted lines in figures 1 and 4. Error bars denote SEM; * significantly different *versus* steady-state of anesthetic effect; + significantly different *versus* isoproterenol in combination with isoflurane plus GDPβS; # significantly different *versus* isoproterenol in combination with halothane (without inhibitor).

Table 1. Shifts in Steady-state Inactivation by Halothane and Isoflurane

	Δ Shift $V_{1/2}$ (mV)		
		+ GDPβS	+ PKI Inhibitor
Halothane	−4.29 ± 0.20* (17)	−2.90 ± 0.35*† (6)	−3.06 ± 0.16*† (11)
Isoflurane	−3.51 ± 0.22* (19)	−2.03 ± 0.27*‡ (8)	−3.32 ± 0.17* (10)

* Indicates significantly different *vs.* control.

† Significantly different *vs.* halothane without inhibitor.

‡ Significantly different *vs.* isoflurane without inhibitor.

Changes in $V_{1/2}$ in the absence and presence of GDPβS or PKI-(6-22)-amide are shown relative to control conditions. Data are corrected for spontaneous background shift (except in the presence of GDPβS) as described in Results section. Number of cells is denoted in brackets.

indirect pathway involves the stimulation of cAMP mediated by protein kinase A (PKA), whose catalytic component can phosphorylate and modulate the functional activity of the Na channel.[11,28] Phosphorylation of the cardiac Na channel is also mediated by protein kinase C.[29] Although recent 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,[20,30] there is considerable evidence of volatile anesthetic action on signal transduction pathways. Consequently, the anesthetic effects on the cardiac Na channel also may be secondary to changes in the second messenger system.

In the present study, the depressant effects of halothane on I_{Na} are significantly diminished, but not completely abolished, in the presence of GDPβS, suggesting the involvement of G proteins. Similar results in the presence of PKI, a PKA inhibitor peptide, suggest that modulation of I_{Na} by halothane may be *via* a G protein, cAMP-dependent pathway. This is further supported by findings that halothane stimulates AC activity by inhibiting the function of the inhibitory G proteins (G_i).[14] Yet the exact nature of the involvement of G proteins in the modulation of I_{Na} by halothane is not clear. Halothane has been shown to produce a dose-dependent inhibition of the stimulatory action of catecholamines on AC but to have no effect on basal, nonstimulated AC activity.[31] In contrast, Anthony *et al.*[15] have found that halothane stimulates the basal AC activity but does not affect stimulation of AC activity produced by either forskolin or isoproterenol.[32] These discrepancies have been attributed to either a loss of some component(s) during membrane isolation or to species differences.[32] In the present study, the anesthetic agent was always applied first followed by forskolin or isoproterenol. Thus the effect of halothane was present before AC stimulation. Therefore, our findings correspond with the results of Anthony *et al.*[15] Because the presence of

Table 2. Shifts in Steady-state Inactivation by Isoproterenol and Forskolin

	Δ Shift $V_{1/2}$ (mV)		
		After Maximal Anesthetic Effect	
	From Control	+ Halothane	+ Isoflurane
Isoproterenol	−2.33 ± 0.34* (7)	−2.29 ± 0.38† (8)	−1.78 ± 0.22† (10)
Isoproterenol + GDPβS	−0.13 ± 0.36‡ (6)	−1.50 ± 0.50† (6)	−0.76 ± 0.15†‡ (10)
Isoproterenol + PKI inhibitor	−1.45 ± 0.16*§ (6)	−2.10 ± 0.11†§ (11)	−0.02 ± 0.43 (10)
Forskolin	−2.08 ± 0.63* (6)	−1.31 ± 0.15† (9)	−1.23 ± 0.16† (9)

* Indicates significantly different *vs.* control.

† Significantly different *vs.* anesthetic effects.

‡ Significantly different *vs.* isoproterenol combined with halothane and GDPβS.

§ Significantly different *vs.* isoproterenol combined with isoflurane and PKI inhibitor.

Changes in $V_{1/2}$ under anesthetic-free conditions are shown relative to control conditions. Changes in $V_{1/2}$ in the presence of anesthetics were determined after the maximal anesthetic effect has occurred. Data are corrected for spontaneous background shift (except in the presence of GDPβS) as described in Results section. Number of cells is denoted in brackets.

GDPβS or PKI inhibitor fragment did not completely abolish the effect of halothane on I_{Na}, a pathway or mechanism beyond G proteins and PKA may also be involved.

In the presence of halothane, isoproterenol produces a disproportionately large decrease in peak inward Na^+ current. Based on the 14.6% effect of isoproterenol alone, we would predict another 8.5% (0.579 × 0.146) additional blockade from the 42.1% blockade observed in the presence of halothane alone. Instead, a 20.1% additional block was observed. An additive effect would have predicted that isoproterenol exerts the same proportional effect in the presence of anesthetics as it does in its absence. This suggests the presence of some sort of synergistic interaction between halothane and β-adrenergic stimulation. Cardiac β-adrenoceptors are coupled through a stimulatory guanine nucleotide binding protein (G_s) to AC.[26] When bypassing the G-protein cascade by using forskolin, which directly stimulates AC, the positive interaction between halothane and isoproterenol is abolished. This finding suggests that a G-protein-dependent but cAMP-independent pathway may be involved in the interaction between halothane and isoproterenol. The direct membrane-delimited pathway, which modulates I_{Na} *via* G_s, might be responsible for the augmented effect of isoproterenol in the presence of halothane. This hypothesis is supported by the result that GDPβS, but not PKI, fully prevented the isoproterenol effect on I_{Na} in the presence of halothane. Thus, even when protein kinase A activity is blocked, the membrane-delimited pathway may be available for I_{Na} modulation. The mechanism by which a G-protein pathway results in the enhanced effect of isoproterenol in the presence of halothane remains unclear. A potential explanation could be either an externalization of β-receptors, resulting in an increased number of β-receptors on the cardiac cell surface or a facilitated β-receptor-G_s protein coupling. However, radioligand studies showing no effects of halothane on binding to cardiac β-adrenoceptors and findings that halothane uncouples rather than facilitates coupling of β-adrenoceptors exclude these possibilities.[33]

Differences in the action of isoflurane and halothane on the cardiac I_{Na} provide evidence of distinct pathways in the modulation of cardiac Na^+ current by these two anesthetics. In contrast to halothane's effect, the suppressive effect of isoflurane on I_{Na} in the presence of PKI in the pipette is unchanged compared with the effect of isoflurane alone. Therefore, isoflurane may not act on I_{Na} *via* a cAMP-dependent pathway. This view is supported by another study showing that isoflurane, in contrast to halothane, has no effect on basal AC activity.[15] However, the depressant effect of isoflurane on I_{Na} is significantly diminished in the presence of GDPβS. Thus the results indicate that isoflurane, like halothane, acts through a G protein-dependent pathway; however, isoflurane action on I_{Na} may involve a cAMP-independent pathway. A possible mechanism of action is that isoflurane acts on cardiac Na channel through the direct, membrane-delimited pathway *via* the G_s protein. Another possible pathway may be protein kinase C.[34]

Another difference between halothane and isoflurane is that the isoproterenol effect is not enhanced in combination with isoflurane. However, surprisingly, when protein kinase A was blocked, the effect of isoproterenol in combination with isoflurane had a small but significant effect on I_{Na}. This is an unexpected result because our findings suggest a cAMP-independent pathway for the effect of isoflurane on I_{Na}. Yet blocking PKA activity reveals some sort of synergistic interaction between isoflurane and isoproterenol. Although this result is puzzling, it suggests that the mechanism involved in the interaction between β-adrenergic stimulation and isoflurane may not be identical to the one involved in the absence of β-simulation. This notion is also supported by the action of halothane, in which the positive interaction with isoproterenol may be *via* a cAMP-independent pathway. In contrast, in the absence of β-adrenergic stimulation, the effect of halothane on I_{Na} involved a PKA pathway.

The effects of anesthetics and β-adrenergic stimulation on I_{Na} cannot be explained by shifts in steady-state inactivation. Similar to the results of Ono *et al.*,[28] the hyperpolarizing background shift in our study was unaffected by PKA inhibition. However, the hyperpolarizing background shift was not evident in the presence of GDPβS. These results provide evidence that G proteins, independent of PKA activity, may be involved in the spontaneous hyperpolarizing shift of steady-state inactivation $V_{1/2}$.[24] Shifts in the presence of halothane and isoflurane, either in the absence or presence of GDPβS or PKI inhibitor fragment, can account only in part for the effects on peak I_{Na}. Furthermore, the shifts in steady-state inactivation were found to be similar between either isoproterenol or forskolin alone and isoproterenol or forskolin in the presence of anesthetics. Thus the positive interaction on suppression of peak I_{Na} between halothane and β-adrenergic stimulation cannot be explained by the shifts in steady-state inactivation. Interestingly, in the presence of GDPβS or PKI, we observed

small but significant differences between shifts in $V_{1/2}$ in the presence of isoproterenol alone and isoproterenol in combination with anesthetics. The significance of these findings is not known.

In the myocardium, volatile anesthetics differ substantially in their ability to potentiate the catecholamines-induced dysrhythmias in patients and laboratory animals, and halothane is more "sensitizing" then either isoflurane or enflurane.[8,35] The cellular mechanism(s) of this catecholamine-sensitizing effect are not completely understood. As previously reported, epinephrine or norepinephrine and halothane have synergistic negative dromotropic effects, slowing conduction of cardiac impulses to a greater extent than does isoflurane.[36,37] Our studies show some type of synergistic interaction between halothane and isoproterenol in decreasing I_{Na}, which may contribute to the prodysrhythmogenic effects of halothane.[8,37]

In summary, the present study provides evidence that intracellular second messenger systems are involved in the regulation of the fast cardiac Na^+ current by volatile anesthetics in ventricular myocytes obtained from adult guinea pig hearts. Furthermore, our results suggest that different mechanisms are responsible for the modulation of I_{Na} by halothane and isoflurane. The effect of halothane involves the signal-transducing G protein that acts through a cytoplasmic, cAMP-dependent pathway and for isoflurane a G protein-dependent but cAMP-independent pathway. Our studies also show that the inhibition of cardiac I_{Na} during β-adrenergic stimulation by isoproterenol is more pronounced in the presence of halothane than isoflurane, indicating some form of synergistic interaction between halothane and isoproterenol.

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