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Isoflurane, but not Halothane, Induces Protection of Human Myocardium via Adenosine A_1 Receptors and Adenosine Triphosphate–sensitive Potassium Channels

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Background: **Volatile anesthetics produce differing degrees of myocardial protection in animal models of ischemia. The purpose of the current investigation was to determine the influence of isoflurane and halothane on myocardial protection in a human model of simulated ischemia and the role of adenosine A_1 receptors and adenosine triphosphate–sensitive potassium (K_{ATP}) channels in the anesthetic pathway.**

Methods: **Human atrial trabecular muscles were superfused with oxygenated Krebs-Henseleit buffer and stimulated at 1 Hz, with recording of maximum contractile force. Fifteen minutes before a 30-min anoxic insult, muscles were pretreated for 5 min with either anoxia, the A1 agonist N^6-cyclohexyladenosine, 1% halothane or 1.2% isoflurane. These treatments were also performed in the presence of either the K_{ATP} channel antagonist glibenclamide or the A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX). Anesthetic effects were also determined on K_{ATP} currents in isolated whole cell voltage-clamped human atrial myocytes.**

Results: **Recovery of force (recorded 60 min after anoxia) in isoflurane-pretreated muscles was reduced from 76.6 ± 7.5% of baseline to 43.7 ± 7.1% by pretreatment with glibenclamide, and to 52.5 ± 6.2% by pretreatment with DPCPX. Halothane treatment provided no cardioprotection and seemed to inhibit protection by anoxic preconditioning. Halothane decreased whole cell K_{ATP} currents in atrial myocytes, whereas isoflurane had no effects.**

Conclusions: **This study demonstrates the cardioprotective effects of isoflurane in contrast to the effects of halothane. Furthermore, A1 receptors and K_{ATP} channels seem to mediate the beneficial effects of anoxia and isoflurane in human myocardium. (Key words: Isolated muscles; myocardial protection; volatile anesthetics.)**

THE intraoperative and perioperative period is a time during which ischemia may be induced in patients by stress[1,2] and also by certain anesthetics.[3–5] The detrimental consequences of myocardial ischemia have been shown to be reduced by pretreatment with transient ischemia, the phenomenon termed "ischemic preconditioning" (IPc).[6] Studies of the mechanism of IPc suggests that protein kinase C (PKC) activity may be enhanced by IPc[7] and, in turn, may activate the adenosine triphosphate–sensitive potassium channel (K_{ATP}).[8,9] Although the sarcolemmal K_{ATP} channel may contribute, recent studies have suggested that the relevant K_{ATP} channel actually resides in the mitochondria.[10,11] Cardioprotective benefits of K_{ATP} channel openers have been observed both *in vivo*[12] and *in vitro*, whereas K_{ATP} channel antagonists such as glibenclamide (glyburide) and 5-hydroxydecanoate preclude the preconditioning effectiveness of ischemia.[13,14]

Like IPc, volatile anesthetics have also been shown to provide protection for ischemic myocardium, both experimentally[15,16] and more recently in a clinical study.[17] One possible common site of action between volatile anesthetics and IPc may be the K_{ATP} channel of cardiac myocytes, because the ability of volatile anesthetics to alter channel activity has been demonstrated in experiments on isolated myocytes.[18] Furthermore, glibenclamide inhibits cardioprotection by isoflurane in a canine model of ischemia.[16] The protection afforded by isoflurane is similarly inhibited by antagonists of adenosine receptors,[19] suggesting that adenosine receptor activation may also be involved in the pathway linking

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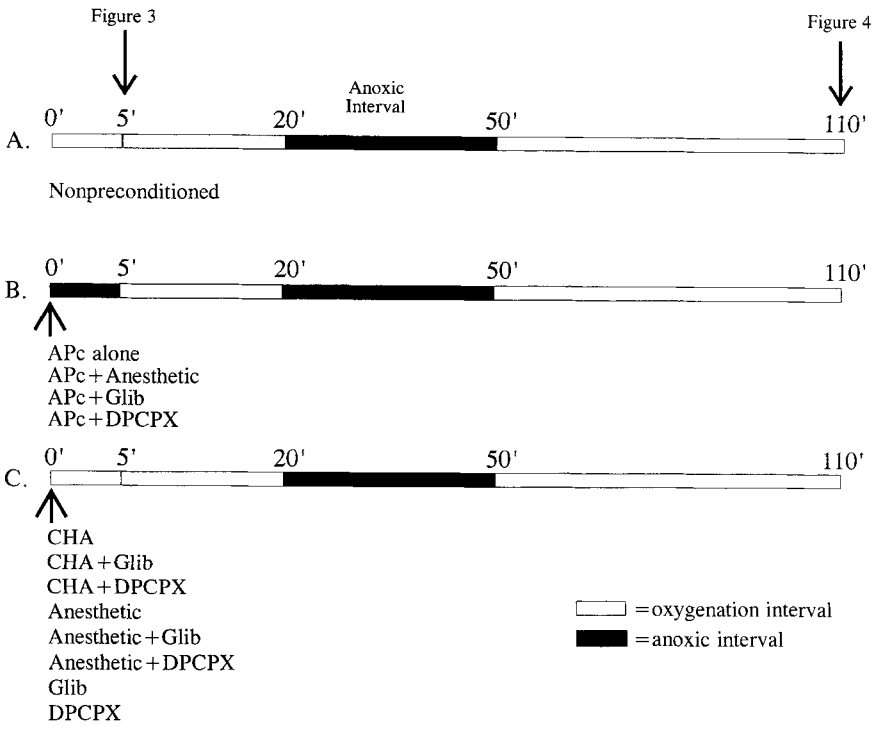

Fig. 1. Experimental protocol for human atrial muscles. Isolated trabeculae were equilibrated in a muscle bath for at least 1 h before application of one of the protocols. Excluding a time control group (not shown), all muscles were made anoxic for 30 min (black area). (*A*) Nonpreconditioned muscles were exposed to 30-min anoxia alone. (*B*) In the absence of oxygen, muscles underwent anoxic preconditioning (APc) alone or in the presence of 0.3 μM glibenclamide (Glib), 10 nM 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), 1% halothane, or 1.2% isoflurane. (*C*) In the presence of oxygen, muscles were treated with 10 nM N^6-cyclohexyladenosine (CHA), 1% halothane, or 1.2% isoflurane in the presence and absence of 0.3 μM glibenclamide or 10 nM (APc + DPCPX, isoflurane + DPCPX) or 100 nM (CHA + DPCPX) DPCPX for 5 min. Time points corresponding to data reported in figures 3 and 4 are denoted by arrows.

isoflurane to cardioprotection. The purpose of the current investigation was to determine any interaction between volatile anesthetics and preconditioning in a human model of simulated myocardial ischemia using anoxia (anoxic preconditioning).

Materials and Methods

Tissue Acquisition and Dissection

Human right atrial appendages were acquired from patients undergoing coronary artery bypass grafting surgery before bypass in accordance with guidelines of the University Human Investigation Committee. Patients taking oral hypoglycemics were excluded from the study. Appendages were transported from the operating room to the laboratory in oxygenated solution containing 130 mM NaCl, 5 mM Na_2SO_4, 6 mM KCl, 5 mM $MgCl_2$, 2 mM NaH_2PO_4, 20 mM glucose, and 5 mM HEPES, and were oxygenated for a further 30 min. Trabeculae were carefully dissected from the appendages and, using 2-mm circular spring clips, were vertically suspended from a force transducer (Model 400; Cambridge Technology, Watertown, MA) in an organ bath maintained at 32°C. This lower temperature is used to prevent automaticity and facilitate pacing. The bath was filled with a Krebs-Henseleit buffer consisting of 118.5 mM NaCl, 4.8 mM KCl, 24.8 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 1.44 mM $MgSO_4$, 2.5 mM $CaCl_2$, 10 mM glucose, and 10 mM pyruvate, pH 7.4, and bubbled constantly with 95% O_2-5% CO_2. Muscles were equilibrated for 10 min before being field stimulated to contract at 0.1 Hz (Grass Stimulator, model S88; Grass Instruments, Watertown, MA) for another 20 min. Muscles were then stretched to the minimum length to produce maximum isometric contractions (L_{max}) and stimulated at 1 Hz until a steady force of contraction was maintained for at least 20 min. The resting tension on muscle and stimulation voltage remained constant for the duration of the experiment. The buffer was changed every 15 min before and during the experiments. Muscles that failed to generate 1 mN of force were not used in the experiments.

Experimental Protocol

After stabilization of contractile performance, muscles were subjected to a 5-min treatment interval (fig. 1). During this treatment interval, muscles were alternatively pretreated with 100 nM N^6-cyclohexyladenosine (CHA; fig. 1C), an adenosine A_1 agonist, or with anoxic preconditioning (APc) by bubbling the organ bath with 95% N_2-5% CO_2 instead of 95% O_2-5% CO_2 (fig. 1B) to simulate ischemia. These pretreatments were also re-

peated in the presence of 0.3 μM glibenclamide, a K_{ATP} channel antagonist, or 10 nM (with APc) or 100 nM (with CHA) 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), an A_1 antagonist, to determine the role of K_{ATP} channels and A_1 receptors, respectively. These concentrations of drugs were similar to those used in previous *in vitro* studies.[14,21,22] DPCPX and glibenclamide treatments were also administered alone as controls.

In anesthetic treatment groups, 1% halothane or 1.2% isoflurane was delivered to the organ chamber for 5 min either in 95% O_2-5% CO_2 (fig. 1C) to determine the effect of prior exposure to anesthetic alone, or in 95% N_2-5% CO_2 (fig. 1B) to determine interaction of anesthetics with preconditioning anoxia. The specific carrier gas was passed through a calibrated anesthetic vaporizer to produce the stated vapor concentration. The vapor concentrations used resulted in aqueous concentrations of 0.23 mM halothane and 0.26 mM isoflurane, which approximates the aqueous concentration at 37°C achieved by 1 minimum alveolar concentration of halothane (0.75%) or isoflurane (1.3%). Anesthetic treatments were also performed in the presence of 0.3 μM glibenclamide and 10 nM DPCPX (fig. 1C). Muscles that served as time control or those subjected only to the subsequent anoxic insult alone (nonpreconditioned) received no treatment during this initial 5-min interval (fig. 1A). At the end of the treatment (time 5 min) and after a washout, a 15-min interval followed during which no treatments were administered. Next, muscles in all groups (except control) were made anoxic by replacing 95% O_2-5% CO_2 with 95% N_2-5% CO_2 in the buffer for 30 min, followed by a 60-min oxygenated recovery period.

The trabeculae that were used in these experiments were 4-5 mm in length with a 1-mm^2 cross-sectional area. The average weight of trabeculae (n = 132) used was 3.11 ± 2.8 mg. Pretreatment force generated in muscles ranged from 1 to 17 mN and averaged 7.35 ± 8.8 mN (table 1). Actual baseline values for developed force did not differ significantly among treatment groups. Some experiments were excluded from the study because of insufficient recovery after treatment. Nine studies were excluded from the APc group, two from the isoflurane in the presence of DPCPX group, and one from the glibenclamide control group.

Table 1. Mean ± SD Values for Pretreatment Force of Contraction of Isolated Human Atrial Trabeculae in Each Separate Treatment Group

Treatment	Force (mg ± SD)
Time control	376.1 ± 693.8
Nonpretreated	885.7 ± 749.9
AP	527.5 ± 528.2
AP + 0.3 μM Glib	885.6 ± 404.3
AP + 10 nM DPCPX	536.2 ± 389.7
AP + 1.2% Isoflurane	632.9 ± 578.2
1.2% Isoflurane	1201.6 ± 1529.5
1.2% Isoflurane + 0.3 μM Glib	1666.6 ± 1893.4
1.2% Isoflurane + 10 nM DPCPX	552.8 ± 288.15
AP + 1% Halothane	713.3 ± 947.9
1% Halothane	491.7 ± 659.0
1% Halothane + 0.3 μM Glib	1140.3 ± 1766.2
10 nM CHA	590.6 ± 244.2
10 nM CHA + 0.3 μM Glib	414.5 ± 332.4
10 nM CHA + 100 nM DPCPX	344.3 ± 256.9

AP = anorexic pretreated; CHA = N^6-cyclohexyladenosine; DPCPX = 8-cyclopentyl-1,3-dipropylxanthine; Glib = glibenclamide.

Atrial Myocyte Isolation

Atrial myocytes were isolated from right atrial appendages using a procedure based on that of Nánási *et al.*[22] Trabeculae minced into 1-mm^3 pieces were allowed to equilibrate for 40 min in a low Ca^{2+} buffer containing 130 mM NaCl, 0.3 mM $CaCl_2$, 5 mM Na_2SO_4, 6 mM KCl, 5 mM $MgCl_2$, 2 mM NaH_2PO_4, 5 mM HEPES, and 20 mM glucose adjusted to pH 7.3 with NaOH. The tissue was then digested for 25-30 min with 1 mg/ml collagenase (Type I; Sigma, St. Louis, MO) and 0.15 mg/ml pronase (Type XIV; Sigma). Initial digestion was followed by a 1-2-min wash in calcium-free solution with 0.25 mg/ml aprotinin (Sigma) adjusted to pH 7.4 with NaOH. A second enzyme solution containing 1 mg/ml albumin, 5 mg/ml Na_2ATP, 0.5 mg/ml creatine, 0.5 mg/ml pyruvate, and 0.5 mg/ml β-hydroxybutyrate with 2 mg/ml collagenase and 0.1 mg/ml elastase (Type III; Sigma) was superfused for 3-12 min. The tissue fragments were gently triturated and filtered through a 250-μm nylon mesh. The myocytes were stored at 4°C in medium (20 mM KCl, 10 mM glucose, 70 mM glutamate, 10 mM β-hydroxybutyrate, 10 mM taurine, 10 mM EGTA, and 1% albumin) for 1 h before recordings were made. Throughout the isolation procedure, all solutions were gassed with 100% O_2 and maintained at 37°C.

Whole Cell Voltage Clamp

The myocyte suspension was placed in a chamber mounted on an inverted microscope and allowed to settle to the bottom. Quiescent rod-shaped myocytes with clear striations were whole cell voltage clamped at room temperature (22-25°C) in external solution consisting of 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 10 mM HEPES, pH 7.4. Microelectrodes were

pulled (Model PB-7; Narishige Instruments, Tokyo, Japan) from glass pipettes and polished with a microforge (CPM2; ALA Scientific, New York, NY). Pipette resistances were typically 3–4 MΩ. Pipette internal solution consisted of 140 mM KCl, 5 mM EGTA, 5 mM HEPES, 10 mM KOH, 1 mM $MgCl_2$, 0.1 mM Na_2ATP, and 0.1 mM NaADP. Currents were evoked by 10-ms voltage steps from −100 to 30 mV from a holding potential of −80 mV. Halothane or isoflurane was bubbled into the extracellular solution *via* air and superfused over the cells in the absence or presence of 50 μM 2,4-dinitrophenol (DNP) to enhance K_{ATP} currents. Halothane administered at vapor concentrations of 0.5%, 1.0%, 1.5%, 2.0%, and 2.5% resulted in aqueous concentrations in the cell chamber of 0.23, 0.51, 0.68, 1.0, and 1.1 mM, respectively. Isoflurane administered at 1.0%, 2.0%, and 3.0% resulted in aqueous concentrations of 0.47, 0.96, and 1.25 mM, respectively. Currents were recorded with pCLAMP 5.0 (Axon Instruments, Foster City, CA) and analyzed using PCS analysis software developed in our laboratory.

Chemicals

Glibenclamide (Sigma) was made as a stock solution in 1:1:1 ethanol, polyethyl glycol, and 1 N sodium hydroxide. DPCPX (RBI, Natick, MA) was made as a stock solution in 95% ethyl alcohol, and CHA (RBI) was made as a stock solution in distilled water.

Statistical Analysis

All data are expressed as mean ± SD percent of the percent pretreatment (time 0 min) force, except as specified in table 1. Anesthetic and preconditioning treatment groups were compared between groups using a one-way analysis of variance and Student-Neuman-Keuls *post hoc* analysis. $P < 0.05$ was considered statistically significant.

Results

Effects of Preconditioning with Anoxia on Human Myocardial Contractility

All data are expressed in the text as a percent of the force of contraction at the initiation (time 0 min) of the experimental protocol. The time course of the changes in force of contraction for APc and anesthetic experiments is shown in figure 2. Contractile force of untreated trabeculae showed a modest decrease during the 110 min of these experiments (fig. 2A) to 82.3 ± 5.0% of the time-0 value. Untreated muscles made anoxic for 30 min and reoxygenated for 60 min contracted with a force of only 30.4 ± 8.3% of pretreatment at the conclusion of the experiment. Pretreatment of the muscles with a single 5-min anoxic interval reduced contractile

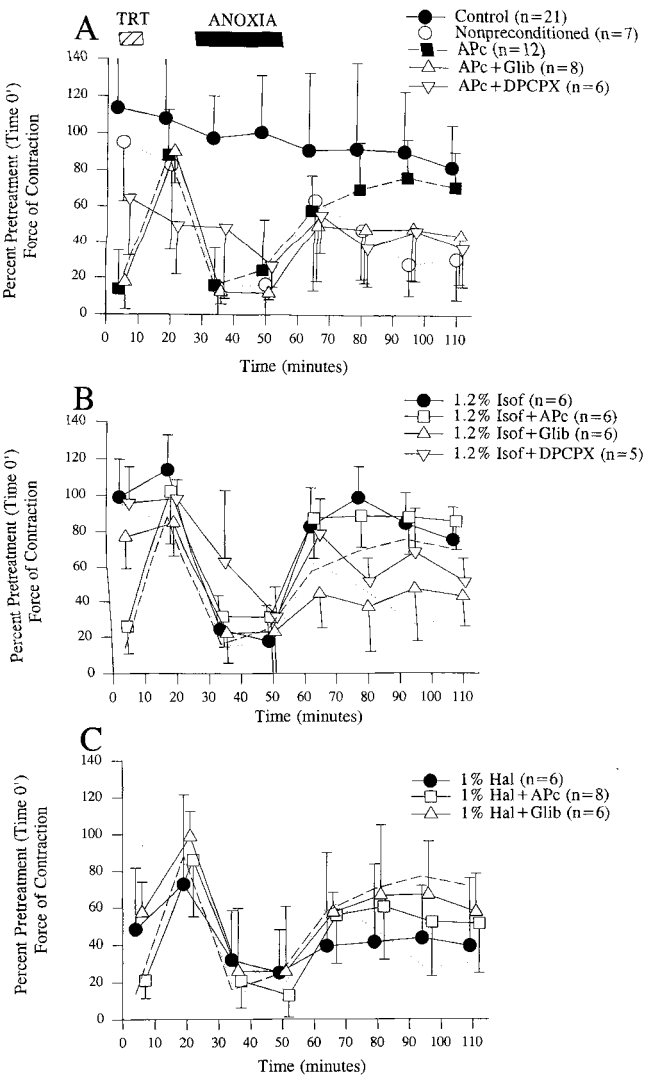

Fig. 2. Time course of effects of pretreatments and anoxia on contractile force in isolated human atrial trabeculae. Mean (± SD) percent of pretreatment (time 0) from the end of the treatment period (time 5 min) until the conclusion of the experiment. The treatment interval (anoxia and/or drug) is indicated by a banded bar, and the filled black bar denotes the interval of anoxia. (*A*) Effects of 0.3 μM glibenclamide (Glib) and 10 nM 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) on anoxic preconditioning (APc). The time control is indicated by a solid line. (*B* and *C*) Preconditioning of muscles with 1.2% isoflurane (Isof) and 1% halothane (Hal). Values for the nonpreconditioned (dotted line) and APc (dashed line) groups are also shown for comparison.

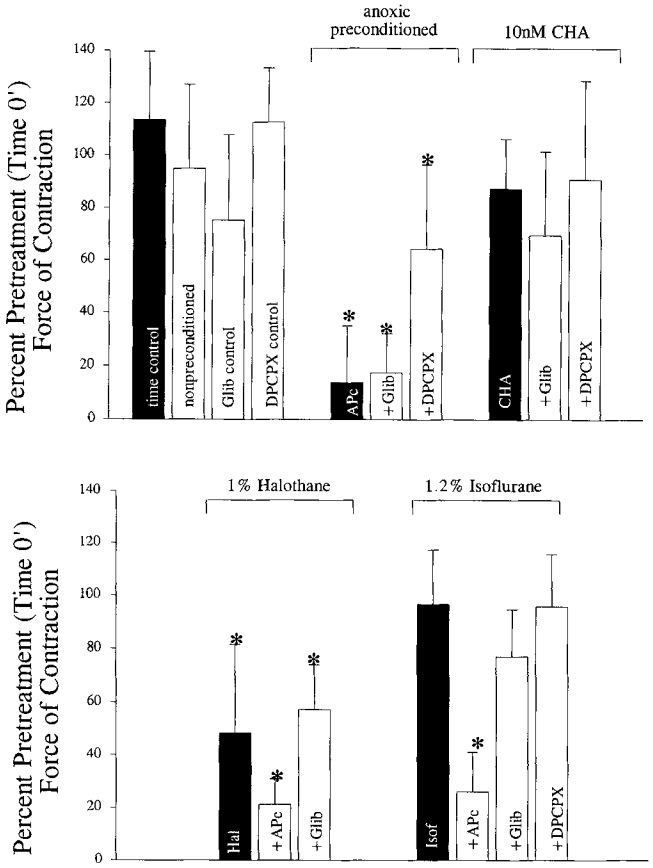

Fig. 3. Alteration in contractile force (expressed as mean ± SD percent of pretreatment force) of human atrial trabeculae at the end of the initial 5 min of the treatment episode for the specific intervention indicated. These data correspond to the first data point in figure 2. (*Top*) No treatment was administered in the time control or anoxia groups. Effects of 0.3 μM glibenclamide (Glib) and 10 or 100 nM 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) on the 5-min treatment with anoxia or 10 nM N^6-cyclohexyladenosine (CHA). (*Bottom*) Effect of 1.2% isoflurane (Isof) and 1% halothane (Hal) on contractile force in the absence and presence of 0.3 μM Glib or 10 nM DPCPX. *$P < 0.05$ as compared with untreated control.

force to less than 25% of pretreatment control but resulted in postanoxic recovery of contractile force to 71.6 ± 5.5% of pretreatment (time 0 min) after 60 min of reperfusion ($P < 0.05$ *vs.* recovery of nonpreconditioned muscles). This protocol was used throughout the remainder of the experiments to determine the relative effects of combining treatments with APc.

Anesthetic Effects on Anoxia and APc

The effects of the volatile anesthetics on contractile force were evaluated at the end of the 5-min treatment interval (fig. 3) and again 60 min postanoxia (110 min; fig. 4) in both untreated and pretreated muscles. When administered in the absence of oxygen, neither anesthetic altered the anoxia-induced decrease in contractile force over the 5-min treatment interval. In the presence of oxygen, 1.2% isoflurane produced no significant change in contractile force. In contrast, delivery of 1% halothane *via* oxygen decreased force of contraction to 48.3 ± 13.6% pretreatment after 5 min. The postanoxic recovery of muscles in these treatment groups also differed. Muscles pretreated with 1.2% isoflurane alone recovered 76.6 ± 7.5% pretreatment control force post-

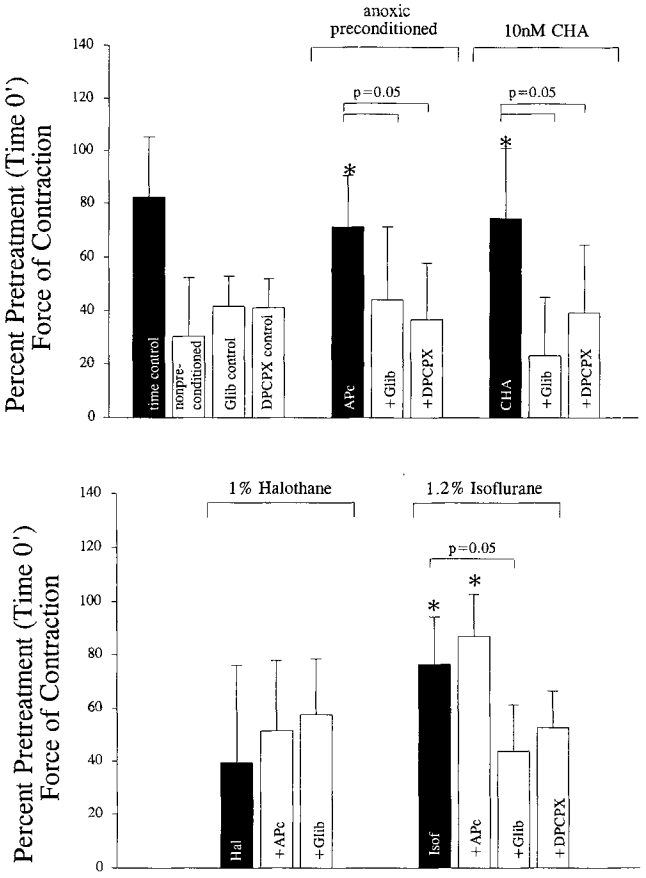

Fig. 4. Recovery of pretreatment control force of contraction (expressed as mean ± SD percent of control, time 0) of human atrial trabeculae 60 min after a 30-min anoxic insult. These data correspond to the final data point in figure 2. (*Top*) Overall recovery of postanoxic force of contraction in the muscles that were preconditioned with anoxia (APc) or 10 nM N^6-cyclohexyladenosine (CHA) in the absence and presence of 0.3 μM glibenclamide (Glib) and 10 or 100 nM 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) are shown. (*Bottom*) Effect of 1.2% isoflurane (Isof) and 1% halothane (Hal) on recovery of postanoxic force. Muscles were pretreated with anesthetics in the absence and presence of 0.3 μM Glib or 10 nM DPCPX. *$P < 0.05$ as compared with nonpreconditioning.

anoxia (110 min; fig. 4), not different from APc muscles. When administered concurrently with APc, 1.2% isoflurane did not alter the degree of cardioprotection provided by APc as assessed after 60-min recovery (fig. 4). In muscles pretreated with 1% halothane alone, force of contraction at the conclusion of the experiments was only 39.6 ± 14.9% pretreatment control. Furthermore, recovery of muscles that were anoxically pretreated in the presence of halothane was significantly decreased compared with APc muscles ($P < 0.05$). Therefore, although 5-min pretreatment with halothane directly decreased force of contraction in isolated muscles (fig. 3), it inhibited the ability of anoxia to precondition myocardium and provide protection after 60-min recovery. In contrast, 1.2% isoflurane had cardioprotective activity similar to that of APc.

Role of Adenosine A_1 Receptors and K_{ATP} Channels

Pretreatment with the A_1 receptor agonist CHA resulted in improved postanoxic recovery (110 min) similar to APc (74.6 ± 8.4%). Postanoxic recovery in APc and CHA-pretreated muscles was similarly inhibited by DPCPX to 39.5 ± 9.6% and 36.9 ± 8.6% of pretreatment control, respectively. These findings corroborate previous studies that suggested a role of A_1 receptors in physiologic preconditioning.[22] When given concurrently with isoflurane pretreatment, 10 nM DPCPX inhibited the postanoxic recovery to 52.5 ± 6.2% of pretreatment control, comparable to that seen in APc muscles. Pretreatment with 0.3 μM glibenclamide to block activation of K_{ATP} channels also inhibited postanoxic recovery in CHA and APc-pretreated muscles (fig. 4), as has been previously demonstrated. This dose of glibenclamide also produced a decrease of postanoxic recovery of force in 1.2% isoflurane-pretreated muscles ($P < 0.05$; figs. 2 and 4). Recovery of halothane-treated muscles was not significantly altered by the inclusion of glibenclamide. Pretreatment with these antagonists alone (glibenclamide, DPCPX; figs. 2 and 4) did not alter the postischemic recovery of contractile force as compared with nonpreconditioned muscles.

Anesthetic Effects on K_{ATP} Current in Isolated Myocytes

Whole cell currents ranged from −198.5 ± 44.5 to 487.5 ± 81.1 pA at voltages from −100 to 30 mV. To determine if either volatile anesthetic was directly activating or inhibiting K_{ATP} channel currents, anesthetics were applied to myocytes in the absence of channel activators or in the presence of DNP. In the absence of DNP-enhanced current, the baseline K^+ conductance was not enhanced by either halothane or isoflurane (n = 6, data not shown). Application of 50 μM DNP augmented whole cell outward currents by 65.4 ± 23.6% (n = 11), with negligible effect on inward current. DNP-induced currents recorded at voltages from −100 to 30 mV ranged from −400 to 1,500 pA. Halothane (1%) decreased inward and outward currents stimulated by DNP at −100 and 30 mV by 29.8 ± 4.5% and 31.9 ± 6.3%, respectively. The results from a representative experiment are shown in figure 5. Increasing concentrations of halothane produced slightly greater decreases in outward current but no further reduction of inward current (table 2). Halothane maximally reduced inward current at a concentration of 1%, whereas 2.5% halothane produced the greatest reduction of outward current observed in these experiments. Isoflurane failed to produce any significant changes in either inward or outward K_{ATP} current (I_{KATP}) when administered in concentrations as high as 3% (table 2). These currents were susceptible to 50-65% inhibition by glibenclamide (data not shown). The high degree of variability observed in the isoflurane-treated groups is insignificant and probably resultant of variations in the whole cell current. This variability was not apparent in halothane-treated cells because of the substantial and consistent effect of halothane treatment.

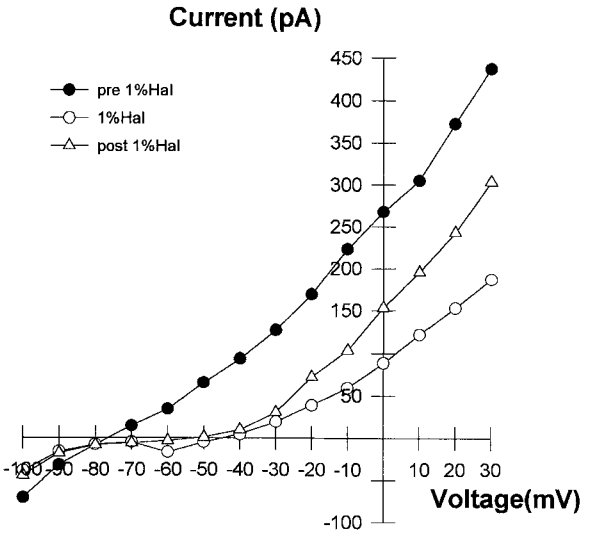

Fig. 5. Representative current–voltage plot depicting current amplitude as a result of command voltage recorded from a single human atrial myocyte. Adenosine triphosphate–sensitive potassium currents stimulated in the presence of 100 μM 2,4-dinitrophenol before anesthetic (black circles), 2 min after washin with 1% halothane (open circles), and 3 min after washout of anesthetic (open triangles).

Table 2. Current in Isolated Human Atrial Myocytes following Treatment with Anesthetics

	Halothane				Isoflurane		
$V_{command}$	0.5% (n = 4)	1.0% (n = 7)	2.0% (n = 7)	2.5% (n = 6)	1.0% (n = 7)	2.0% (n = 5)	3.0% (n = 3)
−100 mV	−8.8 ± 20%	−29.8 ± 11.8%*	−14.2 ± 18.5%	−22.6 ± 26%	−2.9 ± 22.7%	−4.1 ± 16.2%	14.1 ± 52.0%
30 mV	−18.3 ± 23.7%	−31.9 ± 16.7%*	−25.7 ± 16.7%*	−38.4 ± 19.6%*	4.4 ± 8.3%	−7.2 ± 25.2%	39.0 ± 98.8%

Data expressed as mean ± SD of the percent of pretreatment control. Group size indicated in parentheses.

* Denotes $P < 0.05$ compared with pretreatment control.

Discussion

The objectives of this study were to verify studies suggesting protective roles of adenosine receptors and cardiac K_{ATP} channels using this human model of preconditioning, and to determine if volatile anesthetics could pharmacologically imitate or modulate the physiologic effects of APc. These findings support previous reports suggesting that preconditioning in human myocardium involves adenosine receptor activation and K_{ATP} channel activation[14,23] as well as providing evidence toward a mechanism for anesthetic effects in ischemic myocardium.

The anesthetics studied were isoflurane, which represents the most widely used volatile anesthetic despite prior concerns regarding its potential for causing ischemia,[3-5] and halothane, a historically widely applied anesthetic with somewhat greater ability to depress myocardial contractility.[24,25] At clinically relevant concentrations, isoflurane protected the human myocardium in this model against a protracted 30-min anoxia to a similar extent as did hypoxic preconditioning. This effect of isoflurane seems to be mediated through A_1 receptors and the K_{ATP} channel, as the A_1 antagonist DPCPX and the K_{ATP} channel antagonist glibenclamide, respectively, inhibit it. Halothane, at clinically relevant concentrations, provided no benefit to anoxic myocardium and seemed to inhibit the cardioprotection provided by hypoxic preconditioning. In isolated myocytes, halothane inhibited induced K_{ATP} currents, but isoflurane produced no effect.

A human model of simulated ischemia was used to examine the interactions between anesthetics and preconditioning, which have been somewhat characterized in several other species,[15,16] but it remained to be determined whether the effects persisted in the human model. Although experiments performed in a human model have the benefit of more relevant clinical extrapolations, there are a few limitations. For example, the human model possesses a high degree of variability because of possible drug interactions and disease states. Therefore, appendages were not used if obtained from patients currently on an oral hypoglycemic regimen in an attempt to preclude from the study samples with K_{ATP} channels altered by prior pharmacologic interventions. Another consideration was the use of volatile anesthetic in the surgical procedure during which the atrial specimen is removed, because this study attempted to demonstrate anesthetic-specific effects on myocardial protection. Because of its widespread use as a supplementary anesthetic during cardiac surgery at our institution, patients were frequently exposed to isoflurane (0.5–1.0%), which could theoretically precondition the sample. However, studies were initiated at least 90 min (and anoxic insult performed 110–140 min) after removal of the atrium, times that extend beyond the early window of preconditioning.[8] Furthermore, comparison is made with control experiments that were performed in parallel. Nevertheless, our experimental results may be superimposed on some small residual effects. The contribution of endothelial function to a model of protection must also be examined, because myocardial protection mediated by IPc has recently been shown to include not only the myocytes themselves, but also to extend to endothelium.[26] Obviously, protection of the endothelium may be critical to preventing subsequent alterations in perfusion due to disruptions in endothelial regulation of vascular tone. However, anesthetic effects in this model of superfused muscle segments is unlikely involve a major contribution from endothelium. Another issue that must be considered is the temperature at which each experiment was performed. Temperatures below physiologic 37°C have been found to decrease channel responsiveness to potassium channel openers,[27,28] which suggests an alteration in channel activity. This effect could theoretically alter the anesthetic responses in isolated muscles contracting at 32°C and patch-

clamped myocytes at 22–25°C. No studies to date specifically address temperature effects on K_{ATP} channel function. Conducting these experiments at the stated temperatures allows comparison with other studies that have typically been performed at these somewhat subphysiologic temperatures.

Finally, inherent to models of preconditioning is a variability in the methods used to simulate ischemia. In our experiments, ischemia was simulated by anoxia, which was achieved by substitution of nitrogen for oxygen. Although many studies use a duration of anoxia up to 90 min, 30 min has been used in other human models.[29] This anoxic period conceivably may result in a reversible cardiodepression without cell death ("myocardial stunning"), which has been found by some to be resistant to protection from IPc.[30] However, in this study, the cardiodepression by 30-min anoxia was lessened by prior anoxic conditioning. The reason for this discrepancy could be explained by the fact that the current study uses restoration of contractile function, not cell viability, as the measure of postischemic recovery. Nonetheless, because this study focuses on contractile performance, a hypoxic insult sufficient to prevent functioning is adequate for the determination of cardioprotection.

The possible involvement of adenosine A_1 receptors and K_{ATP} channels as mediators of physiologic preconditioning has been suggested by studies in several animal models, including human ones.[14,21,31] It has been suggested that adenosine receptors may be activated by preconditioning and, in turn, activate K_{ATP} channels, possibly through an intermediate such as PKC. Evidence in support of this theory include: (1) adenosine-stimulated preconditioning of human atrial trabeculae can be partially blocked by glibenclamide[23]; (2) IPc of rabbit myocardium enhances translocation of PKC isoforms η and ϵ[7]; (3) preconditioning of human atrial trabeculae with a K_{ATP} channel opener is inhibited by a PKC antagonist[14]; and (4) K_{ATP} currents evoked in rabbit myocytes by metabolic inhibition are increased in the combined presence of PKC activation and adenosine.[32] The current study confirms that adenosine receptors and K_{ATP} channels may sequentially mediate physiologic preconditioning as well as pharmacologic protection provided by anesthetics. Several possible mechanisms by which K_{ATP} channel activation results in cardioprotection against an ischemic insult have been proposed, including a shortening of action potential duration. However, this has been refuted by findings demonstrating protection by potassium channel openers in the absence of action potential effects,[33] suggesting that activation of sarcolemmal K_{ATP} channels may not mediate cardioprotection. Recent studies have described a K_{ATP} channel on the inner membrane of the mitochondria, which is selectively activated by diazoxide and inhibited by 5-hydroxydecanoate.[34] Studies using these agents in isolated rat and rabbit hearts suggest mitochondrial K_{ATP} channels can mediate physiologic preconditioning.[35,36] Our results do not provide any information that would distinguish between a sarcolemmal and mitochondrial effect.

The current study confirms findings in animal models demonstrating a cardioprotective effect of isoflurane anesthesia that is also mediated through adenosine receptors and K_{ATP} channels.[16,19] In a porcine model, coronary vasodilation induced by isoflurane can be inhibited by K_{ATP} channel inhibition with glibenclamide.[37] Previous investigators have suggested that this vasodilation by isoflurane may induce coronary "steal" and thereby induce ischemic changes.[3–5] However, subsequent studies suggest if coronary perfusion pressure is adequately maintained there is no evidence of such steal.[38] This study now documents that in human myocardium, isoflurane can play a direct protective role, as has also been demonstrated in animal models.[16,39,40] It is also noteworthy that the protective effect of isoflurane persists beyond its withdrawal for at least 15 min, similar to the "memory" effects previously reported experimentally[41] and clinically.[17]

To determine whether isoflurane exerts a direct effect on K_{ATP} channels, we applied isoflurane to voltage-clamped human atrial myocytes. In doses as high as 3% (roughly 3 minimum alveolar concentration), glibenclamide-sensitive K_{ATP} currents were not affected, either positively or negatively, by isoflurane. These findings suggest that isoflurane does not have a direct effect on channel activation. Because the activator used here, DNP, activates K_{ATP} currents by decreasing intracellular ATP as well as through a direct channel effect,[42] it is possible that protective activity of isoflurane is exerted at a regulatory site on the channel that is circumvented by the direct action of DNP. For example, in rabbit ventricular myocytes, isoflurane has been shown to decrease channel sensitivity to ATP.[18] Another possibility is that the site of protective action of isoflurane is an upstream intermediate, such as an adenosine receptor. The latter theory is supported by the demonstrated inhibition of isoflurane effects by 10 nM DPCPX. A similar study by Kersten *et al.*[40] found that 0.8 mg/kg of DPCPX in dogs partially inhibits the cardioprotective activity of

isoflurane. If this dose produced the maximal effect of A_1 antagonism, as suggested by the investigators, A_1 receptors may be only one mediator of the isoflurane effect on ischemic recovery, and partial mediation by A3 receptors remains a possibility.

The finding that halothane pretreatment diminishes the protective effects of APc contradicts previous studies that have demonstrated a cardioprotection by halothane.[43] The discrepancy may be a result of the difference in experimental species and illustrates the rationale for conducting these experiments in a human model. This study also supports the hypothesis of a possible interaction of halothane with K_{ATP} channels by two observations: (1) halothane blunts the cardioprotection provided by APc in isolated muscles, which is strongly evidenced to be mediated through K_{ATP} channels; and (2) K_{ATP} currents in voltage-clamped isolated human atrial myocytes were blocked by approximately 30% in the presence of halothane. These observations strongly suggest that halothane affects APc through the inhibition of I_{KATP}, although it would require that its effects extend beyond its immediate period of application. Because inhibition of K_{ATP} current by halothane was not as extensive as that seen with glibenclamide, this less-than-complete inhibition may explain why 1% halothane caused only a partial interference with APc.

In conclusion, our findings demonstrate that the adenosine receptor activation of the K_{ATP} channel, which mediates IPc in human atrium, contributes to the cardioprotective effects of the volatile anesthetic isoflurane. Such a protective effect, which can be elicited even when isoflurane is briefly applied clinically in cardioplegic solution,[17] could contribute to the relatively low incidence of myocardial ischemia observed intraoperatively. Disappearance of protection in the immediate postoperative period might be anticipated as the preconditioning effect declines.[42] Furthermore, we show this property is not present among all volatile anesthetics because halothane did not protect and even seemed to attenuate protection by APc.

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