Anesthesiology
83:353–360, 1995

Interaction of Halothane with Inhibitory G-proteins in the Human Myocardium

Ulrich Schmidt, M.D., Robert H. G. Schwinger, M.D.,* Michael Böhm, M.D.†*

Background: **Halothane has been reported to possess a catecholamine-sensitizing effect in laboratory animals and in anesthetized patients and to enhance the positive inotropic effect of isoproterenol in human papillary muscle strips. The current study was designed to investigate further the underlying subcellular mechanisms on human myocardium, in particular the mechanism of action of halothane on G-proteins.**

Methods: **To investigate the effect of halothane on adenylyl cyclase activity, isoproterenol-, guanylylimidodiphosphate (Gpp(NH)p)-, and forskolin-activated enzyme activities were studied alone and in the presence of halothane in native and manganese-treated membranes. The mechanisms of halothane interaction with inhibitory G-proteins (G_i) were studied in adenosine diphosphate-ribosylation studies with pertussis toxin and immunochemical techniques.**

Results: **Halothane (1%) augmented isoproterenol- and Gpp(NH)p-stimulated adenylyl cyclase activity but had no effect on forskolin-stimulated enzyme activity. Manganese ions inhibited the stimulating effect of isoproterenol and Gpp(NH)p on adenylyl cyclase activity, but the effect of forskolin remained unchanged in control and halothane-treated membranes. In the presence of pertussis toxin, the effect of isoproterenol and Gpp(NH)p on adenylyl cyclase activity was enhanced, but further stimulation by halothane was abolished. Halothane did not influence the attachment of Giα to the membrane. No effect of halothane on adenosine diphosphate-ribosylation of Giα by pertussis toxin was observed.**

Conclusions: **Halothane stimulates adenylyl cyclase activity by inhibiting the function of the inhibitory G-proteins by interfering with the effects of the α subunits or $\beta\gamma$ subunits with the effector. Decreased membrane attachment of Giα in the presence of halothane does not occur. The interaction of α and $\beta\gamma$ subunits is not affected by halothane. Halothane does not impair the binding of pertussis toxin to the Giα-protein. (Key words: Anesthetics, volatile: halothane. Sympathetic nervous system, catecholamines: isoproterenol. Heart: G-proteins; signal transduction.)**

HALOTHANE has been reported to facilitate catecholamine-induced arrhythmias in laboratory animals[1] and in anesthetized patients.[2] A potential mechanism of this catecholamine-sensitizing effect has been reported in experiments *in vitro* on isolated, electrically stimulated preparations obtained from human myocardium.[3] In this study, halothane enhanced the efficacy of the positive inotropic effect of isoproterenol. In rat cerebral cortex and brainstem, uncoupling of muscarinic receptors from G-proteins in the presence of halothane have been reported.[4] Also, in rat myocardial membranes, halothane attenuates the muscarinic inhibition of adenylyl cyclase.[5] As reported recently, an effect on the G-protein adenylyl cyclase complex, namely an inactivation of Giα, appears to be involved in the sensitization of the halothane-treated human myocardium toward catecholamines.[6] However, the mechanism of the interaction of halothane with specific components of the heterotrimeric G-protein α-$\beta\gamma$ complex was not investigated.

The current study was designed to elucidate the particular site of action of halothane on inhibitory G-proteins.

Materials and Methods

Human Myocardial Tissue

Left ventricular myocardium from 13 terminally failing hearts (10 from men and 3 from women; age 51 yr ± 4 yr) was obtained from patients after cardiectomy during cardiac transplantation. All patients gave written informed consent before the operation. Drugs used for general anesthesia were flunitrazepam, or midazolam, fentanyl, and pancuronium bromide with isoflurane. Cardiac surgery was performed on cardiopulmonary bypass during hypothermia. The cardioplegic solution used was a modified Bretschneider solution containing

* Staff Cardiologist.

† Associate Professor of Cardiology.

Received from the Klinik III für Innere Medizin der Universität zu Köln, Köln, Germany. Submitted for publication June 20, 1994. Accepted for publication April 10, 1995. Supported by the Deutsche Forschungsgemeinschaft. Dr. Böhm is a recipient of the Gerhard-Hess and Heisenberg programs of the Deutsche Forschungsgemeinschaft.

Address reprint requests to Dr. Böhm: Klinik III für Innere Medizin, Universität zu Köln, Joseph-Stelzmann-Straβe 9, 50924 Köln, Germany.

(in millimolar units): NaCl 15, KCl 10, $MgCl_2$ 4, histidine HCl 180, tryptophan 2, mannitol 30, and potassium dihydrogen oxoglutarate 1.

Membrane Preparation

Left ventricular myocardial tissue was chilled in 30 ml ice-cold homogenization buffer (10 mM tris(hydroxymethyl)aminomethane [Tris]-HCl, 1 mM ethyldiamine tetraacetic acid, and 1 mM dithiothreitol, *p*H 7.4). Connective tissue was trimmed away, myocardial tissue was minced with scissors and membranes were homogenized with a motor-driven glass-polytetrafluorethylene homogenizer for 1 min. The membrane preparation was then homogenized by hand with a glass-glass homogenizer. The homogenate was spun at 484*g* (JA 20 rotor, Beckmann, Munich, Germany) for 15 min. The supernatant was filtered through four layers of gauze, diluted with an equal volume of KCl (1 M) and stored on ice for 10 min. This suspension was centrifuged at 100,000*g* for 30 min. The pellet was resuspended in 50 volumes incubation buffer (adenosine 5′-triphosphate [ATP] 2.5 mM, $MgCl_2$ 2.5 mM, $KHCO_3$ 1 mM and Tris-HCl 2 mM, *p*H 7.4). and homogenized for 1 min with a glass-glass homogenizer. This suspension was recentrifuged at 100,000*g* for 45 min. The final pellet was resuspended in $KHCO_3$ (1 mM) and stored at −80°C. Storage did not alter the results.

Adenylyl Cyclase Determinations

The activity of adenylyl cyclase was determined in a reaction mixture containing 50 μM [^{32}P]α-ATP (approximately 0.3 μCi/100 μl), 50 mM triethanolamine HCl, 5 mM $MgCl_2$, 100 μM ethyleneglycol-bis-(β-aminoethyl ether) tetraacetic acid, 1 mM 3-isobutyl-1-methylxanthine, 5 mM creatine phosphate, 0.4 mg/ml creatine kinase, and 0.1 mM cyclic adenosine monophosphate at *p*H 7.4 in a final volume of 100 μl. The mixture was preincubated for 5 min at 37°C. The reaction was started by addition of the membrane suspension (30 μg/100 μl). The incubation time was 20 min at the same temperature. Reactions were stopped by the addition of 500 μl of 120 mM zinc acetate. Next, the zinc acetate was neutralized by 600 μl Na_2CO_3 (144 mM). After centrifugation for 5 min at 10,000*g*, 0.8 ml of the supernatant was applied to neutral alumina columns equilibrated with 0.1 M Tris-HCl, *p*H 7.5. The effluent was collected and the [^{32}P]cyclic adenosine monophosphate was determined by measuring radioactivity in a liquid scintillation spectrometer. Halothane was applied with carbogen for 5 min to the membrane suspension and incubated in closed vials for 20 min for measurements of adenylyl cyclase activity. Control membranes were treated with carbogen (95% O_2 + 5% CO_2) only in parallel and handled identically. In all experiments, 5 μM guanylylimidodiphosphate (Gpp(NH)p) was present. In the forskolin experiments, guanine-nucleotides were omitted from the reaction to avoid interferences from activated G-proteins with the catalyst.

[^{32}P]Adenosine Diphosphate-Ribosylation by Pertussis Toxin

[^{32}P]Adenosine diphosphate ([^{32}P]ADP)-ribosylation of Giα by pertussis toxin was performed for 12 h at 4°C in a volume of 50 μl containing Tris-HCl (100 mM) (*p*H 8.0 at 20°C), dithiothreitol (25 mM), ATP (2 mM), guanosine 5′-triphosphate (1 mM), ^{32}P-nicotinamide adenine dinucleotide (oxidized form) (NAD^+) (50 nM; 800 Ci/mmol) and pertussis toxin (10 μg/ml) that had been activated by incubation with dithiothreitol (50 mM) for 1 h at 20°C before the labeling reaction. Samples were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (10% (weight in volume) acrylamide, 16 cm total gel length). Gels were stained with Coomassie Blue and dried before autoradiography was performed. There was a linear relation between the amount of incorporated [^{32}P]ADP-ribose and the protein amounts loaded on the gel.

Pertussis Toxin plus Nicotinamide Adenine Dinucleotide Treatment of Membranes

Pertussis toxin treatment was performed under the same incubation conditions as used for [^{32}P]ADP-ribosylation except that ^{32}P-NAD was replaced by 3 mM NAD in the reaction. After two washings, membranes were subjected to [^{32}P]ADP-ribosylation or determination of adenylyl cyclase activity. Control membranes were subjected to the same incubation conditions except that pertussis toxin was omitted from the medium. The ADP-ribosylation was quantified by using autoradiography.

Immunoblotting

Immunoblotting techniques were performed according to Gierschik *et al.*[7] The polyclonal antiserum MB 1 was raised in rabbits against the carboxyl-terminal decapeptide of retinal transducin α (KENLKDCGLF). After electrophoretic separation, proteins were transferred from the sodium dodecyl sulfate polyacrylamide

gel (10%, 16 cm length) (12
BioRad, Richmond, CA). Unde
immunoreactive Giα band wa
were immersed in 100 ml of 3
posed of Tris-HCl 20 mM and
(TBS) and shaken for 1 h at r
they were incubated in the anti
1) containing 100 μl antiserun
in TBS (24 h, room temperat
washings for 10 min with 100 r
Tween 20, the paper was incu
antibody solution (200 μl per
tirabbit immunoglobulin G
Laboratories, Gaithersburg, MD
washings with 0.05% Tween
transferred to 33 mg nitro blu
of 5-bromo-4-chloro-3-indoly
Tris-HCl (0.1 M) containing Na
(5 mM) at *p*H 8.5. Color de
after 10 min by rinsing with w
was dried between two sheet
the conditions used, this seru
against transducin α, $G_{i\alpha_1}$ an
Giα were evaluated by densito
300 A, BioRad). To test the l
detection system, distinct amo
alyzed, giving a linear rel
amounts loaded on the gel and
of Giα.

Halothane Treatment of M

Halothane was administered
CO_2, Lübeck, Germany) by pa
O_2 + 5% CO_2) and directing
membrane suspension. The
were equilibrated for 5 min.
tered at 1% corresponding to
concentration. Control memb
with halothane and handled i

Determination of Halotha

The concentration of halo
suspension was measured by g
a head space analysis. Separat
capillary column (RTX 1701

Miscellaneous

Protein was determined ac
using bovine serum albumin

gel (10%, 16 cm length) (125 mA, 12 h, Transblot, BioRad, Richmond, CA). Under these conditions, one immunoreactive Giα band was detected. The sheets were immersed in 100 ml of 3% gelatin in buffer composed of Tris-HCl 20 mM and NaCl 500 mM, *p*H 7.5 (TBS) and shaken for 1 h at room temperature. Then they were incubated in the antibody solution (*e.g.*, MB 1) containing 100 μl antiserum in 50 ml of 1% gelatin in TBS (24 h, room temperature, shaker). After two washings for 10 min with 100 ml TBS containing 0.05% Tween 20, the paper was incubated with the second antibody solution (200 μl peroxidase-labeled goat antirabbit immunoglobulin G [Kierkegaard and Perry Laboratories, Gaithersburg, MD]) for 2 h. After repeated washings with 0.05% Tween in TBS, the sheets were transferred to 33 mg nitro blue tetrazolium and 15 mg of 5-bromo-4-chloro-3-indolyl phosphate in 100 ml Tris-HCl (0.1 M) containing NaCl (100 mM) and $MgCl_2$ (5 mM) at *p*H 8.5. Color development was stopped after 10 min by rinsing with water, and nitrocellulose was dried between two sheets of filter paper. Under the conditions used, this serum was strongly reactive against transducin α, Giα$_1$ and Giα$_2$. The amounts of Giα were evaluated by densitometric scanning (Video 300 A, BioRad). To test the linearity of the immunodetection system, distinct amounts of protein were analyzed, giving a linear relation between protein amounts loaded on the gel and immunoreactive signals of Giα.

Halothane Treatment of Membranes

Halothane was administered with a Vapor 19 (Dräger CO, Lübeck, Germany) by passing the carbogen (95% O_2 + 5% CO_2) and directing the output through the membrane suspension. The suspended membranes were equilibrated for 5 min. Halothane was administered at 1% corresponding to 1.25 minimum alveolar concentration. Control membranes were equilibrated with halothane and handled identically.

Determination of Halothane Concentrations

The concentration of halothane in the membrane suspension was measured by gas chromatography using a head space analysis. Separation was done on a 60 m capillary column (RTX 1701, ID 530 μm).

Miscellaneous

Protein was determined according to Lowry *et al.*[8] using bovine serum albumin as standard. Sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed as described by Laemmli.[9]

Materials

Giα/Goα standards were provided by Professor Dr. med. P. G. Gierschik (Universität Ulm, Germany). The second antibody was the affinity purified goat antirabbit immunoglobulin G (peroxidase labeled) from Kierkegaard and Perry Laboratories. Halothane was obtained from Hoechst AG (Frankfurt/Main, Germany). (±)-Isoproterenol HCl was obtained from Boehringer (Ingelheim, Germany). Gpp(NH)p, guanosine 5′-triphosphate, ATP, and creatine kinase were obtained from Boehringer. Pertussis toxin was obtained from Sigma (Deisenhofen, Germany) or List Biological Laboratories (Campbell, CA). Forskolin was donated by Dr. Metzger (Hoechst AG, Frankfurt, Germany). All other compounds used were of analytical or best grade commercially available. Only deionized and twice distilled water was used.

Statistical Evaluation

The data shown are mean ± SEM. Statistical significance was estimated with Student's *t* test for unpaired observations and analysis of variance. A *P* value of less than 0.05 was considered significant.

Results

Halothane has been shown to sensitize the myocardium toward exogenous catecholamines in isolated electrically driven preparations obtained from human hearts.[3] To test the hypothesis of whether an interaction of halothane with the G-protein adenylyl cyclase system is part of this sensitization, the influence of halothane on adenylyl cyclase activity was investigated in human cardiac membrane preparations. Halothane increased basal adenylyl cyclase activity, the efficacy of isoproterenol-stimulated adenylyl cyclase activity (fig. 1A), and the efficacy of the poorly hydrolyzable guanosine 5′-triphosphate analog Gpp(NH)p, which is able to stimulate G-protein α subunits directly (fig. 1B). To test whether the catecholamine-sensitizing effect of halothane is attributable to an effect on adenylyl cyclase regulating G-proteins or to a direct effect on the catalytic subunit of the enzyme, forskolin-stimulated adenylyl cyclase activity was studied alone and in the presence of halothane. As shown in figure 1C, halothane did not affect forskolin-stimulated adenylyl cyclase activity.

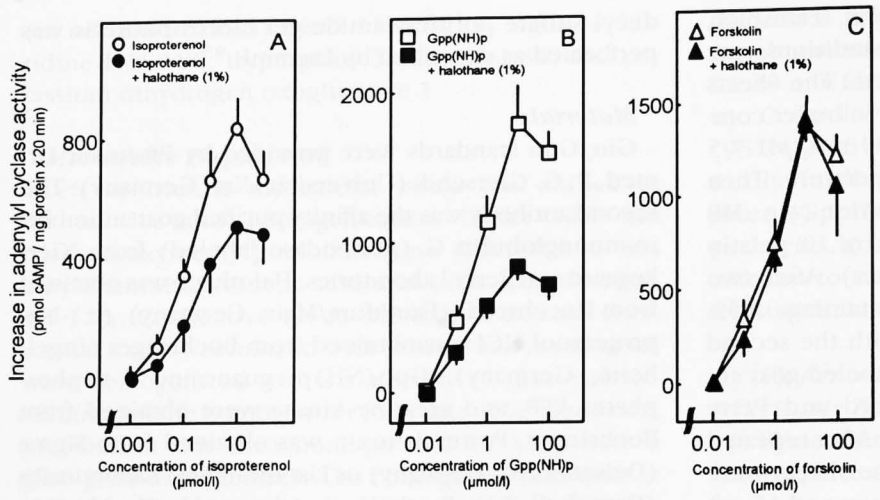

Fig. 1. Concentration-response curve for the effect of (*A*) isoproterenol 0–100 μM (n = 8), (*B*) Gpp(NH)p 0–100 μM (n = 8), or (*C*) forskolin 0–100 μM (n = 8) alone and in the presence of halothane on adenylyl cyclase activity in human myocardial membranes. Ordinates: increase in adenylyl cyclase activity, in picomoles cyclic adenosine monophosphate (cAMP) per milligram protein × 20 min. Abscissas: studied conditions. Basal activities (+ halothane 1%) in picomoles cAMP per milligram protein × 20 min were (*A*) isoproterenol in the presence of Gpp(NH)p 5 μM: 1,009 ± 78 (1,318 ± 71); (*B*) Gpp(NH)p: 641 ± 53 (893 ± 126); and (*C*) forskolin: 652 ± 118 (832 ± 83). Concentration-dependent effects of Gpp(NH)p and forskolin were studied in the absence and those of isoproterenol in the presence of 5 μM Gpp(NH)p. *Significant differences, halothane-treated *versus* native membranes: $P < 0.05$.

However, G-proteins have been reported to influence the forskolin-stimulated adenylyl cyclase activity in S49 lymphoma cells.[10] To study whether the effects of forskolin on adenylyl cyclase activity are a true measure of the catalyst activity under the experimental conditions chosen, experiments with manganese ions were carried out. These ions have been reported to uncouple the catalyst from the regulatory influences of G-proteins.[11,12] The effect of halothane on isoproterenol-, Gpp(NH)p-, and forskolin-stimulated adenylyl cyclase activity were studied in the presence of $MnCl_2$. As shown in figure 2, the stimulatory actions of halothane on isoproterenol- and Gpp(NH)p-stimulated adenylyl cyclase activity were abolished. Numbers of experiments and basal values are given in the legend to figure 2. Taken together, the unchanged effects of forskolin and the augmented effects of Gpp(NH)p in the presence of halothane strongly suggest that halothane interacts with G-proteins but has no effect on the catalytic subunit of adenylyl cyclase.

To study whether halothane affects the function of Giα, adenylyl cyclase activity was determined in control and in pertussis toxin-treated membranes. As shown in figure 3, isoproterenol- and Gpp(NH)p-stimulated adenylyl cyclase activity was augmented in control membranes, whereas the stimulation of halothane was abolished in pertussis toxin-treated membranes, [^{32}P]ADP-ribosylation of pertussis toxin-treated and control membranes with subsequent autoradiography revealed that about 95% of Giα was covalently modified (not shown). These findings are compatible with a pertussis toxin-sensitive inhibition of Giα-function in human heart by halothane.

Further experiments were carried out to specify the mechanisms of this interaction. It has been reported that halothane acts in a nonspecific way by interfering with the lipid bilayer of the membrane.[13] To study whether halothane inhibits the effect of Giα by a non-

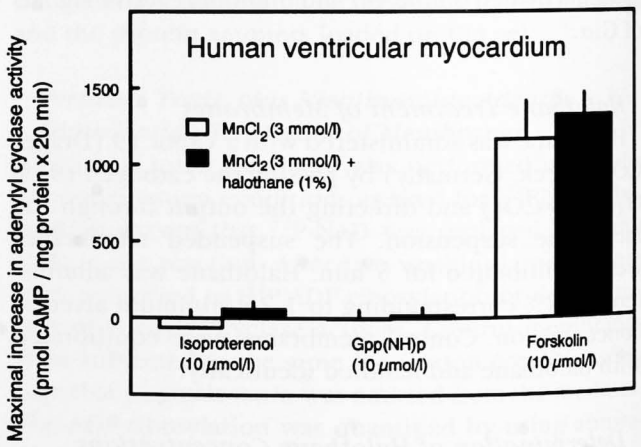

Fig. 2. Effects on adenylyl cyclase activity of isoproterenol (n = 6), Gpp(NH)p (n = 6), and forskolin (n = 6) in the presence of $MnCl_2$ without and with halothane in human myocardial membranes. Ordinate: maximal increase in adenylyl cyclase activity in picomoles cyclic adenosine monophosphate (cAMP) per milligram protein × 20 min. Abscissa: studied conditions. Assays were performed in the absence of $MgCl_2$. Basal adenylyl cyclase activities in control (+ halothane) in picomoles cAMP per milligram protein × 20 min were as follows: isoproterenol: 1,158 ± 190 (1,075 ± 110); Gpp(NH)p: 586 ± 78 (749 ± 86); and forskolin: 745 ± 88 (758 ± 140)

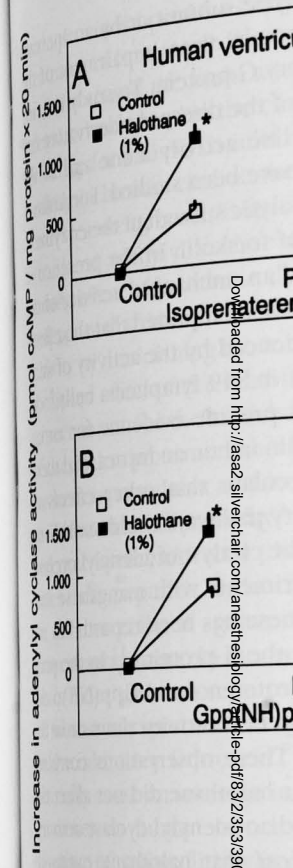

Fig. 3. Increase in adenylyl cycla
and (*right*) pertussis toxin-nicotin
(NAD)-treated human ventricula
with halothane by (*A*) isoproteren
(n = 6) stimulation. Basal adenyly
(+ halothane) membranes in p
monophosphate per milligram pr
40 (1,270 ± 56) in control and 1,4
pertussis toxin plus NAD treatm
cantly increased adenylyl cycla
membranes in the absence of ha
0.01, Gpp(NH)p $P < 0.05$). "$P < 0.0$
untreated membranes.

specific solubilization of th
membrane, a membrane-susp
carbogen plus halothane or
control condition. After centr
Giα was determined in the pa
the supernatant. There was no
of Giα between the membran
plus carbogen or with carbog
To test the hypothesis of wh
with the binding of pertussis t
with the α-$\beta\gamma$ interaction, th
ribose into Giα was measured

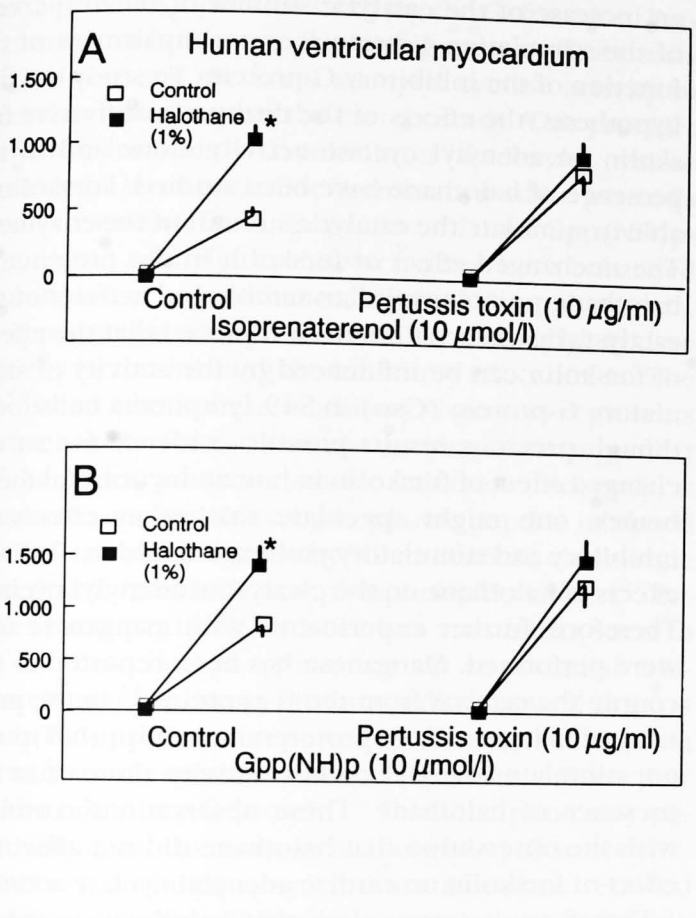

Fig. 3. Increase in adenylyl cyclase activity in (*left*) control and (*right*) pertussis toxin-nicotinamide adenine dinucleotide (NAD)-treated human ventricular membranes without and with halothane by (*A*) isoproterenol (n = 6) and (*B*) Gpp(NH)p (n = 6) stimulation. Basal adenylyl cyclase activities in native (+ halothane) membranes, in picomoles cyclic adenosine monophosphate per milligram protein × 20 min, were 712 ± 40 (1,270 ± 56) in control and 1,423 ± 98 (1,370 ± 104) after pertussis toxin plus NAD treatment. Pertussis toxin significantly increased adenylyl cyclase activity *versus* control membranes in the absence of halothane (isoproterenol $P < 0.01$, Gpp(NH)p $P < 0.05$). *$P < 0.05$, halothane-treated *versus* untreated membranes.

specific solubilization of the Giα-protein from the membrane, a membrane-suspension was treated with carbogen plus halothane or with carbogen alone as control condition. After centrifugation, the content of Giα was determined in the particulate fraction and in the supernatant. There was no difference in the content of Giα between the membranes treated with halothane plus carbogen or with carbogen (fig. 4).

To test the hypothesis of whether halothane interacts with the binding of pertussis toxin to Giα or interferes with the α-$\beta\gamma$ interaction, the incorporation of ADP-ribose into Giα was measured alone and in the presence of halothane. As shown in figure 5, halothane did not influence the efficacy of ADP-ribosylation of Giα. This held true whether the effect of halothane was tested on pertussis toxin catalyzed [^{32}P]ADP-ribosylation of native Giα or whether the efficiency of pertussis toxin plus NAD treatment in the presence of halothane was tested by subsequent [^{32}P]ADP-ribosylation with pertussis toxin.

Discussion

Halothane facilitates the ability of catecholamines to induce cardiac arrhythmias in anesthetized patients.[2] The effects of halothane on the positive inotropic effects of isoproterenol have been studied as a model for the catecholamine-sensitizing effect of the anesthetic.[3] The

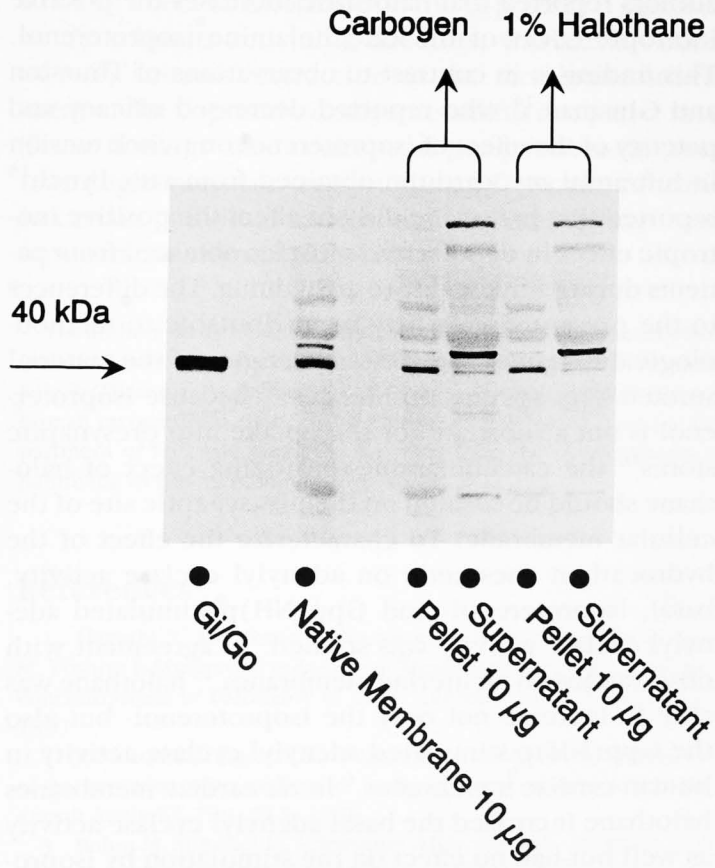

Fig. 4. Representative western blots of Giα in membranes treated with carbogen or with carbogen plus halothane (1%). After centrifugation (10,000*g*) the particular and the soluble fraction were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis before electrophoretic transfer to nitrocellulose membranes. The membranes were incubated with antibodies against the carboxyl terminus of Giα (KENLKDCGLF). Gi/Go α subunits isolated from bovine brain are shown as standards.

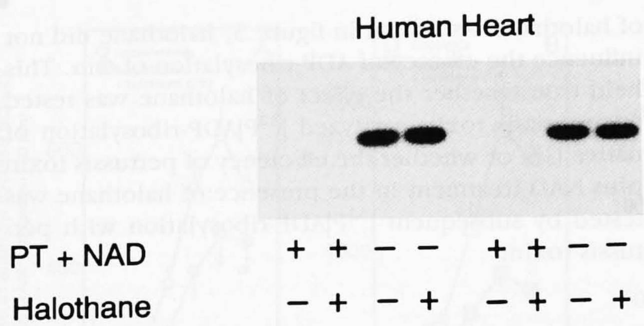

Fig. 5. [^{32}P]Adenosine diphosphate ([^{32}P]ADP)-ribosylation of G-protein α subunits (40 kDa) in control- and halothane (1%)-treated human left ventricular myocardial membranes. After [^{32}P]ADP-ribosylation with pertussis toxin and nicotinamide adenine dinucleotide (NAD) membranes were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis before autoradiography. Each lane contained 10 μg membrane protein.

authors reported that halothane increases the positive inotropic effect of the catecholamine isoproterenol. This finding is in contrast to observations of Thurston and Glusman,[14] who reported decreased efficacy and potency of the effect of isoproterenol on twitch tension in left atrial myocardium obtained from rats. Lynch[15] reported that halothane did not affect the positive inotropic effect in depolarized muscles obtained from patients during surgery due to arrhythmia. The differences to the presented data may be attributable to methodologic differences, the different origins of the material studied,[16] or species differences.[17] Because isoproterenol is not a substrate for the uptake into presynaptic stores[18] the catecholamine-sensitizing effect of halothane should be located on the postsynaptic site of the cellular membrane. To characterize the effect of the hydrocarbon anesthetic on adenylyl cyclase activity, basal, isoproterenol- and Gpp(NH)p-stimulated adenylyl cyclase activity was studied. In agreement with observations on rat uterine membranes,[19] halothane was able to increase not only the isoproterenol- but also the Gpp(NH)p-stimulated adenylyl cyclase activity in human cardiac membranes.[6] In rat cardiac membranes halothane increased the basal adenylyl cyclase activity as well but had no effect on the stimulation by isoproterenol.[5] From the current observations, it appears that halothane possesses direct effects on the G-protein adenylyl cyclase complex, independent from β-adrenoceptors.

Adenylyl cyclase activity is dually regulated by stimulatory and inhibitory G-proteins.[20,21] Therefore, halothane could activate adenylyl cyclase activity either by an increase of the catalytic subunit or by an increase of the stimulating G-protein or an impairment of the function of the inhibitory G-protein. To study the first hypothesis, the effects of the diterpene derivative forskolin on adenylyl cyclase activity alone and in the presence of halothane have been studied. Forskolin is able to stimulate the catalytic subunit of the enzyme.[10] The unchanged effect of forskolin in the presence of halothane points toward an unchanged activity of the catalyst. However, it has been reported that the effects of forskolin can be influenced by the activity of stimulatory G-protein (Gsα) in S49 lymphoma cells.[22] Although previous results provide evidence for an unchanged effect of forskolin in human myocardial membranes, one might speculate that other effects on inhibitory and stimulatory pathways could mask direct effects of halothane on the catalyst of adenylyl cyclase. Therefore, further experiments with manganese ions were performed. Manganese has been reported to uncouple the catalyst from the G-proteins.[12] In the presence of manganese, isoproterenol and Gpp(NH)p did not stimulate adenylyl cyclase activity alone or in the presence of halothane. These observations correlate with the observation that halothane did not affect the effect of forskolin on cardiac adenylyl cyclase activity.

Therefore, it seems clear that halothane exerts its stimulating effect on adenylyl cyclase activity by an effect on the stimulatory G-protein or a functional desensitization of inhibitory G-protein α subunits or an inhibition of the effects of $\beta\gamma$ subunits on effectors. Hypothetical effects of halothane on Gsα proteins were addressed in a previous study.[6] The stimulatory effect on adenylyl cyclase was similar in S49 *cyc* lymphoma cells, which genetically lack Gsα as compared with S49 wild-type cell membranes.[6] Thus, an effect of halothane on Gsα function seems to be very unlikely. In addition, reconstitution of recombinant Gsα from transformed *Escherichia coli* into S49 *cyc* membranes did not alter the effect of halothane compared with native membranes.[6]

The α subunits of heterotrimeric inhibitory G-proteins are subject to the covalent modification by pertussis toxin of a cysteine at the fourth position from the carboxyl terminus.[23] This modification functional inactivates Giα resulting in a decreased inhibition of the basal and receptor modulated adenylyl cyclase activity.[24,25] Treatment of cardiac membranes with pertussis toxin leads to an increased adenylyl cyclase activity.[26] The authors of the latter study suggested that treatment of myocardial membranes with pertussis

toxin provides a pool for studying the Gi function. Pretreatment of human cardiac membranes with pertussis toxin increased the basal adenylyl cyclase activity in previous work[26] and in the current study. This provides evidence for a tonic Gi inhibition of adenylyl cyclase activity in human cardiac membranes. After pertussis toxin treatment, basal, isoproterenol- and Gpp(NH)p-stimulated adenylyl cyclase activities were enhanced and the effect of halothane abolished. From these data, it appears that halothane stimulates adenylyl cyclase activity by diminishing the inhibitory effect of Giα. The abolished effect of the inhibitory G-protein represents a mechanism for the catecholamine-sensitizing effect of the hydrocarbon anesthetic halothane. Recently, it has been reported that a hydrophobic peptide inhibited the activation of Gi or Go by M2 muscarinic cholinergic receptor in reconstituted phospholipid vesicles.[27] Besides these peptides, hydrocarbon anesthetics seem to be another class of agents that act as potential Giα-protein antagonists. In rat myocardium as well as in rat brain halothane was reported to disrupt the coupling of muscarinic serotonin 1a and adenosine A1 receptor to the inhibitory G-protein.[28] This disruption may potentially cause an increase in adenylyl cyclase activity. However, in human myocardium halothane reduced binding of quinuclidinylbencylate to muscarinergic cholinergic receptors but did not affect the receptor coupling to Giα or the negative inotropic effect of carbachol.[3] The unchanged coupling of inhibitory receptors in the presence of an altered Giα function may be due to the receptor G-protein stoichiometry. The number of Giα proteins exceeds the number of M-cholinergic receptors in human myocardium about 1,000-fold. This may explain the differences to cat heart or brain where an interruption of receptor G-protein coupling was observed previously.[4,5,28–30]

Halothane has been suggested to act in a nonspecific way by producing interfacial dehydration of phospholipid membranes.[13] To test the hypothesis of whether pretreatment with halothane facilitates the solubilization of the inhibitory G-protein and its loss from the membrane compartment, resulting in loss of its inhibitory activity on adenylyl cyclase activity, the amount of Giα in the soluble and the membrane phase before and after treatment with halothane was investigated. The unchanged "membrane-attachment" after the treatment of myocardial membranes with halothane strongly argues against this possibility.

The abolished effect of halothane on adenylyl cyclase activity in the presence of pertussis toxin may head the assumption that halothane influences the ADP-ribosylation with pertussis toxin. However, in human cardiac membranes, halothane had no effect on the ADP-ribosylation with pertussis toxin. Therefore, it is likely that the anesthetic does not interact with the binding of pertussis toxin at the α subunit of the G-proteins. Isolated βγ subunits augment the [^{32}P]ADP-ribosylation with pertussis toxin,[31] because the heterotrimeric guanosine diphosphate-bound α-βγ complex is the best substrate for the ADP-ribosylation with pertussis toxin.[32] The unchanged ADP-ribosylation in the presence of halothane argue that halothane may not affect the interaction between α and βγ subunits of the inhibitory G-proteins.

We conclude that halothane exerts its catecholamine-sensitizing effect by interference with the inhibitory G-protein followed by stimulation of adenylyl cyclase activity. The mechanism of action of halothane appears to be an effect of halothane on the G-protein α subunits or βγ subunits activating effectors, whereas an effect of α-βγ subunit interaction or the membrane attachment of the G-protein α subunits seems not play a role. These findings raise conceptual perspectives on pharmacologic intervention to modulate G-protein function in certain pathologic states, such as heart failure[33] and autonomous thyroid adenoma.[34]

The authors thank Elisabeth Ronft and Judith Sabo for their excellent technical assistance. They thank colleagues from the Herzchirurgische Klinik (Director, Professor Dr. B. Reichart) for their support in using human myocardium. They thank Professor M. v. Meyer from the Department of Forensic Medicine for measuring the concentration of halothane in the membrane preparation.

References

1. Hayashi Y, Sumikawa K, Yamatodani A, Kamibayashi T, Kuro M, Yoshiya I: Myocardial epinephrine sensitization with subanesthetic concentrations of halothane in dogs. ANESTHESIOLOGY 74:134–137, 1991

2. Johnston RR, Eger EI II, Wilson C: A comparative interaction of epinephrine with enflurane, isoflurane and halothane in man. Anesth Analg 55:709–712, 1976

3. Bohm M, Schmidt U, Schwinger RHG, Bohm S, Erdmann E: Effects of halothane on β-adrenoceptors in human myocardium: Radioligand binding and functional studies. J Cardiovasc Pharmacol 21:296–304, 1993

4. Aronstam RS, Anthony BL, Dennison RL Jr: Halothane effects on muscarinic acetylcholine receptor complexes in rat brain. Biochem Pharmacol 35:667–672, 1986

5. Narayanan TR, Confer RA, Dennisson RL Jr, Anthony BL, Aronstam RS: Halothane attenuation of muscarinic inhibition of adenylate cyclase in rat heart. Biochem Pharmacol 37:1219–1223, 1988

6. Bohm M, Schmidt U, Gierschik P, Schwinger RHG, Bohm S, Erdmann E: Sensitization of adenylate cyclase by halothane in human myocardium and S49 lymphoma wild-type and cyc- cells: Evidence for an inactivation of the inhibitory G protein (Giα). Mol Pharmacol 45:380–389, 1994

7. Gierschik P, Codina J, Simons C, Birnbaumer L, Spiegel A: Antisera against a guanine nucleotide binding protein from retina cross-react with the β-subunit of the adenylyl cyclase-associated guanine nucleotide binding proteins, N_s and N_i. Proc Natl Acad Sci U S A 82: 727–731, 1985

8. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurements with the folin phenol reagent. J Biol Chem 193:265–275, 1951

9. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685, 1970

10. Seamon KB, Daly JW: Forskolin: Its biological and chemical properties. Advances in Cyclic Nucleotide Phosphorylation Research 20:1–150, 1989

11. Limbird LE, Hickey AR, Lefkowitz RJ: Unique uncoupling of the frog erythrocyte adenylate cyclase system by manganese: Loss of hormone and guanine nucleotide-sensitive enzyme activities without loss of nucleotide-sensitive, high affinity agonist binding. J Biol Chem 254:2677–2683, 1979

12. Cech SY, Broaddus WC, Maguire ME: Adenylate cyclase: The role of magnesium and other divalent cations. Mol Cell Biochem 33: 67–92, 1980

13. Tsai YS, Ma SM, Nishimura S, Ueda I: Infrared spectra of phospholipid membranes: Interfacial dehydration by volatile anesthetics and phase transition. Biochim Biophys Acta 1022:245–250, 1990

14. Thurston TA, Glusman S: Halothane myocardial depression: Interaction with the adenylate cyclase system. Anesth Analg 76:63–68, 1993

15. Lynch C: Effects of halothane and isoflurane on isolated human ventricular myocardium. ANESTHESIOLOGY 68:429–432, 1988

16. Schwinger RHG, Bohm M, Pieske B, Erdmann E: Different β-adrenoceptor-effector coupling in human ventricular and atrial myocardium. Eur J Clin Invest 21:443–451, 1991

17. Bohm M, Diet F, Pieske B, Erdmann E: Screening of positive inotropic agents in isolated cardiac preparations from different sources. J Pharmacol Methods 699:33–44, 1989

18. Callingham BA, Burgen AS: The uptake of isoprenaline and noradrenaline by the perfused rat heart. Mol Pharmacol 2:37–42, 1966

19. Triner L, Vulliemoz Y, Verosky M: The action of halothane on adenylate cyclase. Mol Pharmacol 13:976–979, 1977

20. Gilman AG: G proteins and dual control of adenylyl cyclase. Cell 36:577–579, 1984

21. Boege F, Neumann E, Helmreich EJM: Structural heterogeneity of membrane receptors and GTP-binding proteins and its functional consequences for signal transduction. Eur J Biochem 199:1–15, 1991

22. Darfler FJ, Mahan LC, Koachman AM, Insel PA: Stimulation of forskolin of intact S49 lymphoma cells involves the nucleotide regulatory protein of adenylate cyclase. J Biol Chem 257:11901–11907, 1982

23. Gilman AG: G-proteins: Transducers of receptor-generated signals. Annu Rev Biochem 56:615–649, 1987

24. Milligan G: Techniques used in the identification and analysis of pertussis toxin-sensitive guanine nucleotide binding proteins. Biochem J 255:1–13, 1988

25. Hazeki O, Ui M: Modification by islet-activating protein of receptor-mediated regulation of cyclic AMP accumulation in isolated rat heart cells. J Biol Chem 256:2856–2862, 1981

26. Feldman AM, Cates AE, Veazey WB, Hershberger RE, Bristow MR, Baughman KL, Baumgartner WA, Van Dop C: Increase of the 40,000-molecular weight pertussis toxin substrate (G protein) in the failing human heart. J Clin Invest 82:189–197, 1988

27. Mukai H, Munekata E, Higashijma T: G protein antagonists: A novel hydrophobic peptide competes with the receptor for G protein binding. J Biol Chem 267:16237–16243, 1992

28. Martin DC, Dennison RL, Introna RP, Aronstam RS: Influence of halothane on the interactions of serotonin 1a and adenosin A1 receptors with G proteins in rat brain membranes. Biochem Pharmacol 42:1313–1316, 1991

29. Antony BL, Dennison RL, Aronstam RS: Disruption of muscarinic receptor-G protein coupling is a general property of liquid volatile anesthetics. Neurosci Lett 99:191–196, 1989

30. Baumgartner MK, Dennison RL, Narayan TK, Aronstam RS: Halothane disruption of α2-adrenergic receptor-mediated inhibition of adenylate cyclase and receptor G-coupling in rat brain. Biochem Pharmacol 39:223–225, 1990

31. Watkins PA, Burns DL, Kanaho Y, Liu TY, Hewllett EL, Moss J: ADP-ribosylation of transducin by pertussis toxin. J Biol Chem 260: 1378–1382, 1985

32. Casey PJ , Graziano MP, Gilman AG: G protein $\beta\gamma$ subunits from bovine brain and retina: Equivalent catalytic support of ADP-ribosylation of α subunits by pertussis toxin but differential interactions with Gsα. Biochemistry 28:611–616, 1989

33. Bohm M, Gierschik P, Jakobs KH, Pieske B, Schnabel P, Ungerer M, Erdmann E: Increase of Giα in human hearts with dilated but not ischemic cardiomyopathy. Circulation 82:1249–1265, 1990

34. Selzer E, Wilfing A, Schilferer A, Herrmann M, Grubeck-Lobenstein B, Freissmuth M: Stimulation of human thyroid growth via the inhibitory guanine nucleotide binding (G) protein Gi: Constitutive expression of the G-protein α subunit Giα-1 in autonomous adenoma. Proc Natl Acad Sci U S A 90:1609–1613, 1993

Anesthesiology
83:361–373, 1995

Differential Effe Aortic Input Im element Windke

Douglas A. Hettrick, Ph.D.,* Paul S

Background: Systemic vascular r
aortic pressure [AP] and mean ao
not completely describe left ventri
of the phasic nature of pressure a
impedance (Z_{in}) is an established
LV afterload that incorporates
characteristics and viscoelastic pr
tem. Z_{in} is most often interpreted t
known as the three-element Win
examined the effects of isoflurane
troprusside (SNP) on Z_{in}. Changes
three variables derived from the
aortic impedance (Z_c), total arteri
arterial resistance (R).

Methods: Sixteen experiments w
chronically instrumented for mea
maximum rate of change in left v
diocardial segment length, and AQ
recorded in the conscious state an
at 1.25, 1.5, and 1.75 minimum al
isoflurane and halothane. Z_{in} spec
spectral analysis of AP and AQ w
the phase responses of the transf
lated as the mean of Z_{in} between 2
between Z_{in} at zero frequency an
termined using the formula: C = (A
where A_d = diastolic AP area; MA
mean AQ, respectively; and P_{es} an
diastolic AP, respectively. Param

* Biomedical Engineer, Departmen

† Assistant Professor, Department

‡ Professor, Departments of Anes
Medicine (Division of Cardiology);
partment of Anesthesiology.

Received from the Departments of
and Medicine, Medical College of W
partment of Veterans Affairs Medical
Submitted for publication Decembe
lication April 10, 1995. Supported
Service grant HL 32911, Anesthesiol
08377, and Department of Veterans

Address reprint requests to Dr. W
siology, MEB, Room 462C, Medical C
ierrown Plank Road, Milwaukee, Wis