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Sensitization of Adenylate Cyclase by Halothane in Human Myocardium and S49 Lymphoma Wild-type and *cyc*$^-$ Cells: Evidence for Inactivation of the Inhibitory G Protein $G_{i\alpha}$

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SUMMARY

Halothane has been reported to sensitize the myocardium towards the effects of exogenous catecholamines in patients and laboratory animals. This study was aimed at investigating the catecholamine-sensitizing effects of halothane as well as the underlying subcellular mechanisms in human myocardium. Halothane augmented the positive inotropic effect of isoprenaline but not of Ca^{2+}. The increase of the effect of isoprenaline by halothane was more pronounced in failing myocardium, with increased G_i, than in nonfailing donor hearts. Halothane (1%) increased basal as well as isoprenaline-, NaF-, cholera toxin-, and guanylylimidodiphosphate [Gpp(NH)p]-stimulated adenylate cyclase in human myocardial membranes ($p < 0.05$). Treatment of membranes with pertussis toxin increased adenylate cyclase by 40% and abolished the effect of halothane. Halothane had no effect on forskolin-stimulated adenylate cyclase. The same results, i.e., a pertussis toxin-sensitive increase of adenylate cyclase stimulation by halothane, were obtained in S49 *cyc*$^-$, wild-type, or recombinant $G_{s\alpha}$-reconstituted *cyc*$^-$ cell membranes. Carbachol-stimulated guanosine-5′-*O*-(3-[^{35}S]thio)triphosphate binding was not influenced by halothane, but halothane attenuated the inhibition of adenylate cyclase by Gpp(NH)p in S49 *cyc*$^-$ cells. These data show that halothane stimulates adenylate cyclase and sensitizes adenylate cyclase after stimulation by β-adrenoceptor agonists and guanine nucleotides due to an impairment of $G_{i\alpha}$ function. This mechanism may play a role in the halothane sensitization of myocardial adenylate cyclase towards catecholamines.

General anesthetics like halothane depress synaptic neurotransmission in various areas of the brain (1). The mechanism of this anesthetic effect is understood incompletely and might result from the interaction of inhalational anesthetics with specific proteins (2) or from biophysical changes in the lipid interface of the membrane (3), or both. Several reports indicate that disruption of receptor-G protein coupling in the brain is a general phenomenon of hydrocarbon anesthetics (4). Uncoupling of muscarinic receptors from G proteins in rat cerebral cortex and brainstem (5), direct interactions with forebrain A_1 adenosine receptors and hippocampal serotonin 5-hydroxytryptamine type 1A receptors (6), and depression of α_2-adrenoceptor-mediated adenylate cyclase inhibition (7) have been observed with halothane. However, data on the effects of hydrocarbon anesthetics like halothane on G protein-receptor interactions in other excitable tissues like the myocardium are sparse. In the myocardium, halothane has been reported to sensitize adenylate cyclase to the effects of catecholamines (8–10). This catecholamine-sensitizing effect, which occurs even at subanesthetic concentrations (11), has been suggested to be involved in the arrhythmogenicity observed in patients (10). The underlying mechanism of action has been hitherto unknown. This study aimed at investigating whether halothane is able to sensitize the adenylate cyclase in human heart and at characterizing the underlying mechanism of action.

Materials and Methods

Human myocardial tissue. Left ventricular myocardium from patients with terminal heart failure (n = 19; 17 men and two women; age, 47 ± 3 years) was obtained from patients after cardiectomy during cardiac transplantation. The preoperative diagnosis for all patients was dilated cardiomyopathy. All patients gave written informed consent before the operation. Drugs used for general anesthesia were fentanyl, pancuronium bromide with isoflurane, and either flunitrazepam or

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ABBREVIATIONS: MAC, minimum alveolar concentration; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; Gpp(NH)p, guanylylimidodiphosphate; GTPγS, guanosine-5′-*O*-(3-thio)triphosphate; $rG_{s\alpha}$, recombinant G protein α subunits.

midazolam. Cardiac surgery was performed with cardiopulmonary bypass with hypothermia. Myocardial tissue from three nonfailing donors (two men and one woman; age, 26 ± 4 years) was studied for comparison. The patients' histories and two-dimensional echocardiography studies revealed no evidence of heart disease. The hearts could not be transplanted for technical reasons.

Isolated cardiac preparations and measurement of force of contraction. Immediately after excision, the papillary muscle strips and trabeculae were placed in ice-cold pre-aereated modified Tyrode solution and were delivered to the laboratory within 10 min. Each native papillary muscle was split into thin strips with the muscle fibers running approximately parallel to the length of the strips. The muscles were suspended in an organ bath (75 ml) that was maintained at 37° and contained a modified Tyrode solution of the following composition (in mM): NaCl, 119.8; KCl, 5.4; $MgCl_2$, 1.05; $CaCl_2$, 1.8; $NaHCO_3$, 22.6; NaH_2PO_4, 0.42; glucose, 5.0; ascorbic acid, 0.28; EDTA, 0.05. The bathing solution was continuously aerated with 95% O_2/5% CO_2. The muscles were stimulated by two platinum electrodes using field stimulation from a Grass S88 stimulator (Grass, Quincy, MA) (frequency, 1 Hz; impulse duration, 5 msec; intensity, 10–20% greater than threshold). The resting force was kept constant throughout the experiment. The developed force was measured isometrically with an inductive force transducer (W. Fleck, Mainz, Germany) attached to either a Hellige Helco Scriptor (Hellige, Freiburg, Germany) or Gould recorder (Gould, Cleveland, OH). Preparations were allowed to equilibrate for at least 90 min, with the bathing solution bubbled with carbogen (95% O_2/5% CO_2) being changed once after about 45 min. Concentration-response curves for isoprenaline (0.001–1 μM) or Ca^{2+} (1.8–15 mM) were determined by adding the drugs cumulatively to the organ bath after equilibration of the previous effects (force of contraction stable for 5 min). Each muscle was used for one concentration-response curve only. Halothane was applied with a Vapor 19 (Dräger Co., Lübeck, Germany) to the carbogen (95% 0_2/5% $C0_2$) for the organ baths. In previous experiments to characterize the negative inotropic effects of halothane, concentrations of 1% (94.05% O_2/4.95% CO_2), 2% (93.1% O_2/4.9% CO_2), 3% (92.15% O_2/4.85% CO_2), and 4% (91.2% O_2/4.8% CO_2) (v/v), giving 1.25 MAC, 2.5 MAC, 3.75 MAC, and 5 MAC of halothane, respectively, were studied. Ca^{2+} and isoprenaline effects were studied in the presence of 2% halothane, giving a decline of force of contraction of about 35%. Halothane plus carbogen was applied 20 min before isoprenaline or Ca^{2+}. The negative inotropic effect of halothane was rapidly reversible (3–4 min) after withdrawal of the agent.

S49 lymphoma cells. S49 lymphoma cells (wild-type and *cyc*$^-$) were grown in suspension culture in RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum (culture volume, 100 ml) or 10% (v/v) horse serum (culture volume, 100 ml), 44 mM $NaHCO_3$, 5.5 mM glucose, 5 mM L-glutamine, nonessential amino acids, 1 mM sodium pyruvate, 50 units/ml penicillin, and 50 μg/ml streptomycin, in a humidified atmosphere of 90% air/10% CO_2, as described elsewhere for HL-60 cells (12). The cell density was maintained at approximately 1×10^6 cells/ml. Cells ($1–2 \times 10^{10}$ cells in 10–20 liters of medium) were harvested by centrifugation in a Beckman type JA-10 rotor at 1000 × *g* for 20 min at 4°. The pellets were resuspended in 50 ml of 10 mM triethanolamine HCl (pH 7.4 at 20°). The final pellet was resuspended in 100–150 ml of lysis buffer containing 0.25 M sucrose, 20 mM Tris·HCl (pH 7.5 at 20°), 1.5 mM $MgCl_2$, 1 mM ATP, 3 mM benzamidine, 1 μM leupeptin, 1 mM phenylmethylsulfonyl fluoride, and 2 μg/ml soybean trypsin inhibitor. Cells were homogenized by nitrogen cavitation. The cavitate was centrifuged in a JA-20 rotor at 1500 × *g* for 45 sec at 4°, to remove unbroken cells and nuclei, and was filtered through two layers of cheesecloth. A crude membrane fraction was isolated from the resulting supernatant by centrifugation in a JA-20 rotor at 5000 × *g* for 20 min at 4°. The membranes were washed three times with buffer containing 20 mM Tris·HCl (pH 7.5 at 20°), 1 mM EDTA, 1 mM dithiothreitol, 3 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, 10 μM leupeptin, and 2 μg/ml soybean trypsin inhibitor, resuspended in this buffer to 10 mg of protein/ml, and stored at −80°. The yield of membrane protein was approximately 100 mg/10^{10} cells.

Preparation of $rG_{s\alpha}$. *Escherichia coli* harboring the full length DNA of $rG_{s\alpha}$ was provided by Drs. M. Linder and A. G. Gilman (University of Texas, Dallas, TX). $rG_{s\alpha}$ were expressed in *E. coli* as described by Graziano *et al.* (13). Partial purification was performed according to the method of Lin and Cheng (14).

Reconstitution of S49 *cyc*$^-$ cell membranes with $rG_{s\alpha}$. Reconstitution assays were performed according to the method of Hiroshi and Gilman (15). In brief, $rG_{s\alpha}$ were quantified by [^{35}S]GTPγS binding. Proteins were incubated at 30° for 90 min with [^{35}S]GTPγS in 50 mM triethanolamine HCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol, pH 7.4. Reconstitution was performed for 10 min on ice, with gentle vortexing.

Adenylate cyclase determinations. Particulate washed membrane fractions (10,000 × *g* sediment) were prepared from homogenates of human hearts. The activity of adenylate cyclase was determined in a reaction mixture containing 50 μM [^{32}P]ATP (approximately 0.3 μCi/100 μl), 50 mM triethanolamine HCl, 5 mM $MgCl_2$, 100 μM EGTA, 1 mM 3-isobutyl-1-methylxanthine, 5 mM creatine phosphate, 0.4 mg/ml creatine kinase, and 0.1 mM cAMP, pH 7.4, in a final volume of 100 μl. The mixture was preincubated for 5 min at 37°. The reaction was started with the membrane suspension (heart, 30 μg/100 μl; S49 membranes, 20 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 with 600 μl of 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, pH 7.5. The effluent was collected and the [^{32}P]cAMP was determined by measuring radioactivity in a liquid scintillation counter. Halothane was applied with carbogen to the membrane suspension for 5 min and the mixture was incubated in a closed vial for 20 min for measurements of adenylate cyclase activity. Control membranes were treated in parallel with carbogen only and were handled identically. In all experiments 10 μM Gpp(NH)p was present. In the forskolin experiments, guanine nucleotides were omitted from the reaction to avoid interference of activated G proteins with the catalyst.

[^{32}P]ADP-ribosylation by pertussis toxin. [^{32}P]ADP-ribosylation of $G_{i\alpha}$ by pertussis toxin was performed for 12 hr at 4° in a volume of 50 μl containing 100 mM Tris·HCl (pH 8.0 at 20°), 25 mM dithiothreitol, 2 mM ATP, 1 mM GTP, 50 nM [^{32}P]NAD^+ (800 Ci/mmol), and 10 μg/ml pertussis toxin that had been activated by incubation with 50 mM dithiothreitol for 1 hr at 20° before the labeling reaction. Samples were subjected to SDS-PAGE (10%, w/v, acrylamide; 16-cm total gel length). Gels were stained with Coomassie Blue and dried before autoradiography was performed.

[^{32}P]ADP-ribosylation by cholera toxin. $G_{s\alpha}$ was labeled by cholera toxin-catalyzed ADP-ribosylation with [^{32}P]NAD as substrate. The reaction mixture (50 μl) contained 100 mM potassium phosphate, pH 7.5, 1 mM ATP, 10 mM thymidine, 10 mM arginine, 2.5 mM $MgCl_2$, 5 μg of cholera toxin (preactivated by incubation with 20 mM dithiothreitol at 30° for 10 min), 100 μM GTP, $5–10 \times 10^5$ cpm of [^{32}P]NAD, and 20–160 μg of membrane proteins. After incubation at 37° for 1 hr, samples were subjected to SDS-PAGE and autoradiography as described above.

Pertussis toxin plus NAD 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 adenylate cyclase activity. Control membranes were subjected to the same incubation conditions except that pertussis toxin was omitted from the medium.

Agonist-stimulated GTPγS binding. Binding assays were performed in an incubation mixture of 100 μl containing 0.3 nM [^{35}S]GTPγS, 50 mM triethanolamine, 1 mM dithiothreitol, 1 mM EDTA, 5

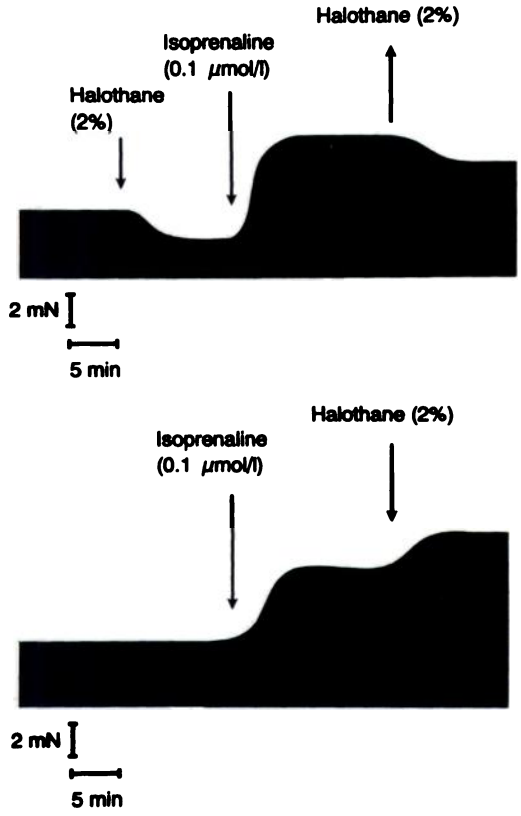

Fig. 1. Original recording of the effects of halothane (2%) and isoprenaline (0.1 μM) on the force of contraction of isolated electrically driven ventricular preparations (1 Hz, 37°, 1.8 mM Ca^{2+}) from human ventricular myocardium. Halothane produced negative inotropic effects alone, but its withdrawal from the carbogen mixture in the presence of isoprenaline reduced the force of contraction (*upper*). Addition of halothane in the presence of isoprenaline increased developed tension (*lower*).

mM $MgCl_2$, and 100 mM NaCl, at pH 7.4. Reactions were started by addition of the membrane suspension (30 μg) in the same buffer and incubation was performed for 30 min at 30°. The incubation was terminated by rapid vaccum filtration through nitrocellulose filters and three washes with ice-cold buffer containing 50 mM Tris·HCl, pH 7.4, 1 mM EDTA, 100 mM NaCl, and 5 mM $MgCl_2$. Nonspecific binding was defined as [^{35}S]GTPγS binding in the presence of 100 μM Gpp(NH)p and was usually <1% of the total [^{35}S]GTPγS in the assay. The standard deviation of the means was typically <5%. Halothane was applied as in the experiments on adenylate cyclase activity.

Miscellaneous methods. Protein was determined according to the method of Lowry *et al.* (16), using bovine serum albumin as the standard. SDS-PAGE was performed as described by Laemmli (17).

Materials. Halothane was from Hoechst AG (Frankfurt/Main, Germany). (±)-Isoprenaline HCl was from Boehringer (Ingelheim, Germany). Gpp(NH)p, GTP, ATP, and creatine kinase were from Boehringer (Mannheim, Germany). Pertussis toxin was 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 grade or the best grade commercially available. Only deionized, twice-distilled water was used throughout.

Statistical evaluation. The data shown are means ± standard errors. Statistical significance was estimated with Student's *t* test for unpaired observations and analysis of variance. A *p* value of <0.05 was considered significant. The drug concentration producing 50% of the maximum effect (EC_{50}) was graphically determined in each individual experiment.

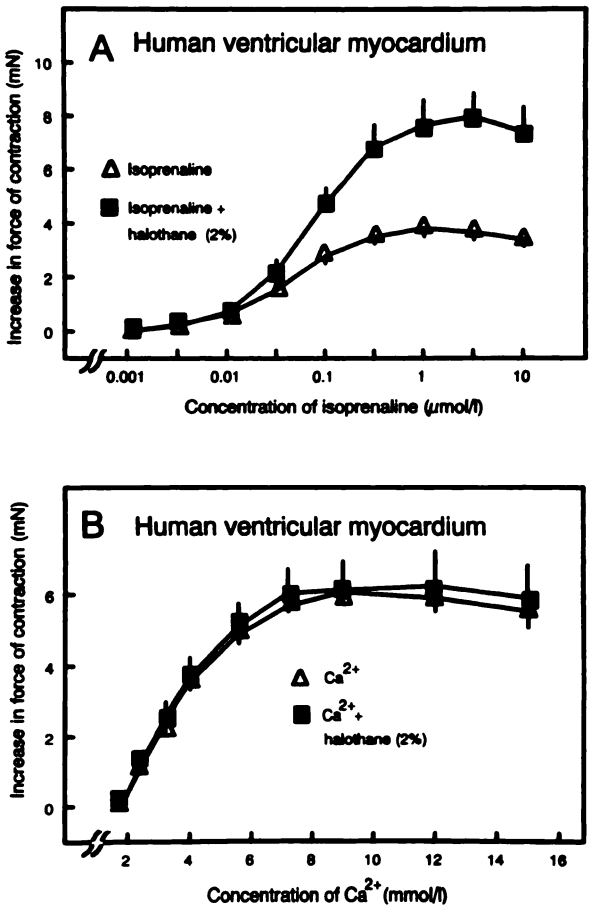

Fig. 2. Concentration-response curves for the effects of isoprenaline (0.001–1 μM, n = 16) (A) and of the increase of the extracellular Ca^{2+} concentration (1.8–15 mM, n = 18) (B), alone and in the presence of halothane, on the force of contraction in isolated, electrically driven, ventricular preparations. *Ordinates*, increase in force of contraction. *Abscissae*, concentrations of isoprenaline or Ca^{2+}. Basal force of contraction was 4.2 ± 0.5 mN (n = 17) in control and 2.9 ± 0.4 mN (n = 17) in halothane-exposed preparations.

Results

Force of contraction. Fig. 1 shows original recordings illustrating the effects of halothane on the isometric force of contraction. Halothane at 2% produced negative inotropic effects alone, but its withdrawal from the gas mixture also reduced the force of contraction in the presence of isoprenaline (Fig. 1, *upper*). Consistent with the latter observation, halothane produced an increase in the force of contraction when applied in the presence of isoprenaline (Fig. 1, *lower*). Thus, halothane produced opposite effects on basal and isoprenaline-stimulated force of contraction. In Fig. 2, the concentration-response curves for the positive inotropic effects of isoprenaline (Fig. 2A) and an increase of the extracellular Ca^{2+} concentration (Fig. 2B) are shown. The experiments were performed in the presence of 2% halothane, which produced a decline in basal force of contraction of 25–35%. Halothane increased the positive inotropic effect of isoprenaline. This held true when increases were evaluated as percentages of basal force of contraction (data not shown) or in absolute values (Fig. 2). In contrast, the positive intropic effects of Ca^{2+} were similar under control conditions and in the presence of halothane (Fig. 2B). To study whether inactivation of $G_{i\alpha}$ could be involved, myo-

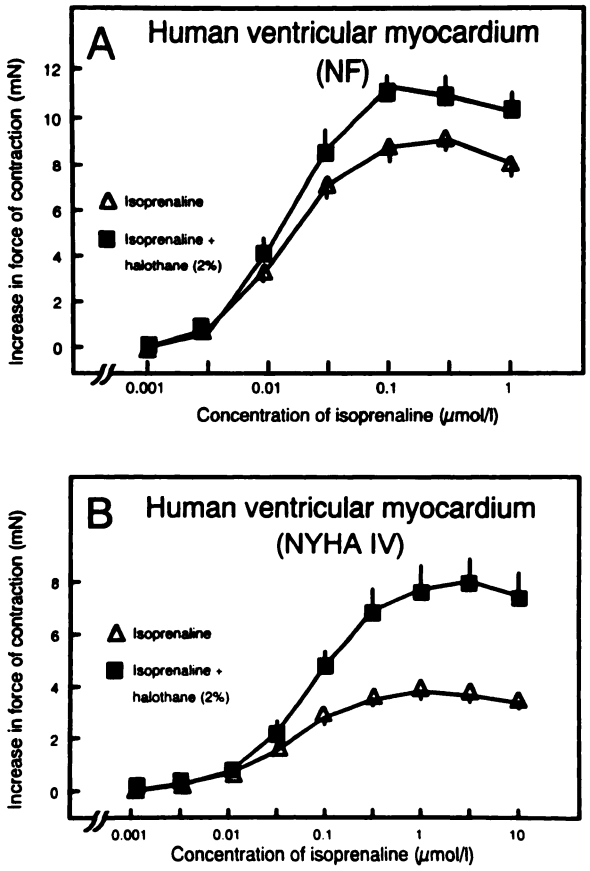

Fig. 3. Concentration-response curves for the effects of isoprenaline (0.001–1 μM) or isoprenaline in the presence of halothane on the force of contraction in isolated, electrically driven, ventricular preparations for nonfailing (*NF*) (A) or failing (*NYHA IV*) (B) human myocardium. *Ordinates*, increase of force of contraction. *Abscissae*, concentrations of isoprenaline. Basal force of contraction was 4.7 ± 0.9 mN (n = 4) in control and 2.9 ± 0.3 mN (n = 4) in halothane-exposed preparations (nonfailing) or 3.1 ± 0.3 mN (n = 9) in control and 2.5 ± 0.3 mN (n = 9) in halothane-exposed preparations (failing).

cardia from failing and nonfailing hearts were compared (Fig. 3). $G_{i\alpha}$ levels were different in the two groups (nonfailing, 1.6 ± 0.3 μg of transducin equivalents/mg of protein, n = 3; failing, 3.1 ± 0.4 μg of transducin equivalents/mg of protein, n = 5). Similar data were reported in more detail in previous work (18). The increase of the positive inotropic effect of isoprenaline was more pronounced in failing myocardium, with higher $G_{i\alpha}$ content (Fig. 3B), than in nonfailing myocardium (Fig. 3A).

Adenylate cyclase activity. To investigate whether the sensitizing effects of halothane on β-adrenoceptor-stimulated force of contraction are due to direct effects on β-adrenoceptor-stimulated adenylate cyclase activity, we measured the enzyme activity in cardiac membranes equilibrated with halothane plus carbogen or carbogen alone (as control). Fig. 4A shows that halothane increased isoprenaline-stimulated adenylate cyclase activity. Similar results were obtained with the poorly hydrolyzable guanine nucleotide Gpp(NH)p (Fig. 4B) and NaF (Fig. 4C), which are able to stimulate G proteins directly. From these data it is conceivable that the sensitizing effect of halothane in human ventricular myocardium could be due to effects of the compound either on the catalyst or on the G proteins regulating adenylate cyclase activity. To test the former hypothesis, we investigated the effects of halothane on forskolin-stimulated adenylate cyclase activity in the absence of guanine nucleotides,

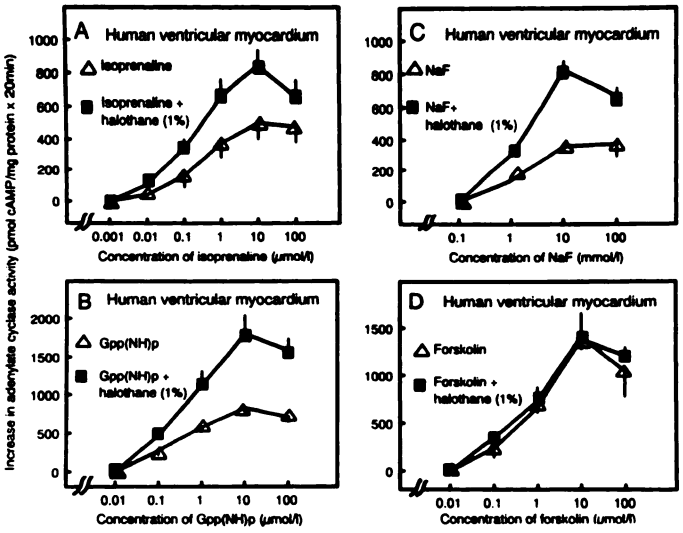

Fig. 4. Concentration-response curves for the effects of isoprenaline (0.001–100 μM) (A), Gpp(NH)p (0.01–100 μM) (B), NaF (0.1–100 mM) (C), or forskolin (0.01–100 μM) (D), alone and in the presence of halothane. *Ordinates*, increase in adenylate cyclase activity. *Abscissae*, concentrations of studied agents. Basal activities (n = 5–8) were as follows: A (isoprenaline), 1009 ± 78 pmol of cAMP/mg of protein × 20 min (plus halothane, 1318 ± 71 pmol of cAMP/mg of protein × 20 min); B [(Gpp(NH)p], 641 ± 53 pmol of cAMP/mg of protein × 20 min (plus halothane, 893 ± 126 pmol of cAMP/mg of protein × 20 min); C (NaF), 1103 ± 101 pmol of cAMP/mg of protein × 20 min (plus halothane, 1678 ± 173 pmol of cAMP/mg of protein × 20 min); D (forskolin), 652 ± 118 pmol of cAMP/mg of protein × 20 min (plus halothane, 832 ± 83 pmol of cAMP/mg of protein × 20 min). Concentration-dependent effects of Gpp(NH)p and forskolin were studied in the absence of Gpp(NH)p and those of isoprenaline and NaF were studied in the presence of 10 μM Gpp(NH)p.

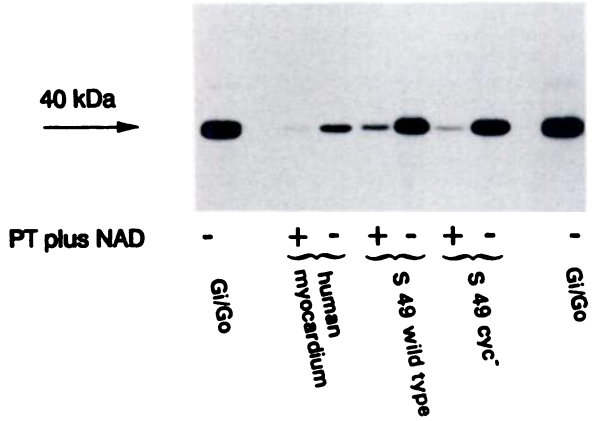

Fig. 5. [^{32}P]ADP-ribosylation of G protein α subunits (40 kDa) in membranes from human myocardium, S49 wild-type cells, and S49 *cyc*$^-$ cells, after treatment with pertussis toxin (*PT*) plus NAD (3 mM) and under the respective control conditions. Membranes were treated with pertussis toxin plus NAD as described in Materials and Methods. Samples (5 μg of S49 *cyc*$^-$ or S49 wild-type cells, 20 μg of human myocardium) were [^{32}P]ADP-ribosylated with pertussis toxin and [^{32}P]NAD and were separated by SDS-PAGE before autoradiography. G_i/G_o α subunits isolated from bovine brain and [^{32}P]ADP-ribosylated with pertussis toxin and [^{32}P]NAD are shown as standards (*rightmost* and *leftmost lanes*).

which is a measure of the catalyst (19). As shown in Fig. 4D, halothane had no effect on the stimulation of adenylate cyclase by forskolin.

Taken together, these data indicate that halothane augmented the stimulatory effects of isoprenaline, the poorly hydrolyzable guanine nucleotide Gpp(NH)p, or NaF to stimulate

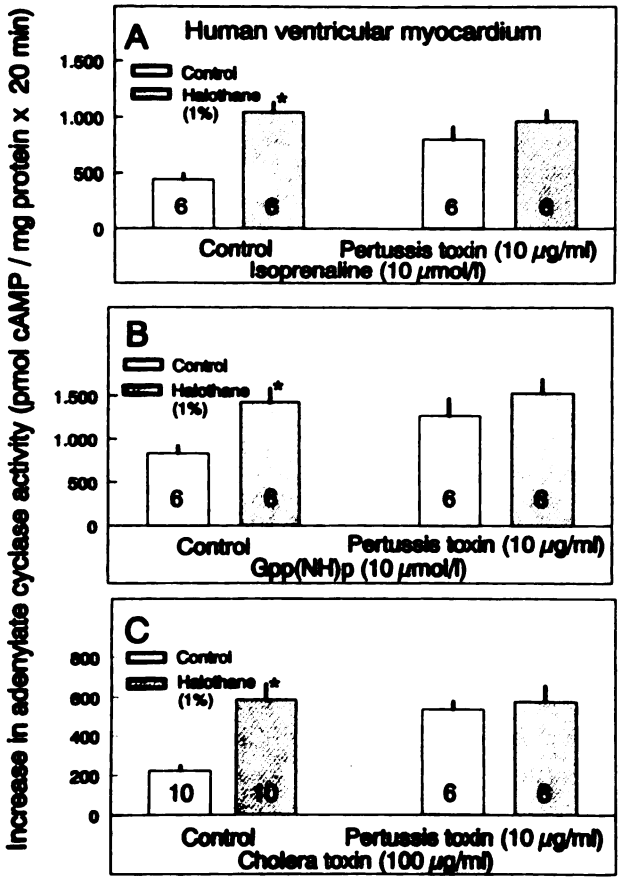

Fig. 6. Increase in adenylate cyclase activity in control (*left*) and pertussis toxin- plus NAD-treated (*right*) human ventricular membranes, without (□) and with (▨) halothane, by isoprenaline (A), Gpp(NH)p (B), and cholera toxin (C) stimulation. Basal adenylate cyclase activity in native (or halothane-treated) membranes was 712 ± 40 (1270 ± 56) pmol of cAMP/mg of protein × 20 min in control and 1423 ± 98 (1370 ± 104) pmol of cAMP/mg of protein × 20 min after pertussis toxin plus NAD treatment. *, $p \leq 0.05$, halothane-treated versus untreated membranes. Membranes were treated as described in Materials and Methods. Pertussis toxin significantly increased adenylate cyclase activity versus control membranes in the absence of halothane (isoprenaline, $p < 0.01$; Gpp(NH)p, $p < 0.05$; cholera toxin, $p < 0.01$). *Numbers in the columns*, numbers of independent experiments performed with triplicate determinations.

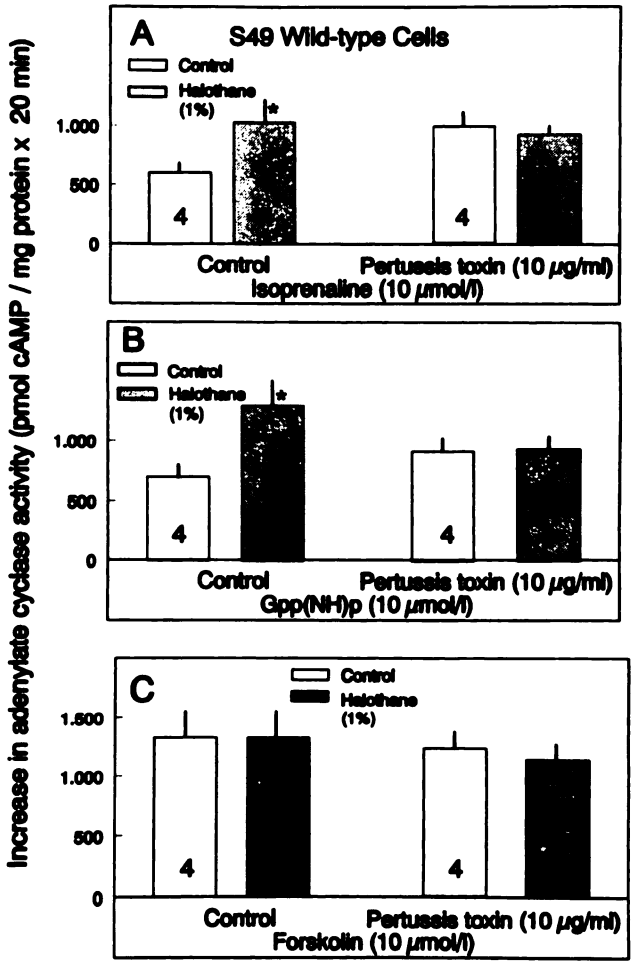

Fig. 7. Increase in adenylate cyclase activity in control (*left*) and pertussis toxin-treated (*right*) S49 wild-type cell membranes, without (□) and with (▨) halothane, by isoprenaline (A), Gpp(NH)p (B), and forskolin (C) stimulation. Basal adenylate cyclase activity in native (or halothane-treated) membranes was 1180 ± 83 (2039 ± 89) pmol of cAMP/mg of protein × 20 min in control and 1798 ± 29 (1723 ± 92) pmol of cAMP/mg of protein × 20 min after pertussis toxin plus NAD treatment. *, $p \leq 0.05$, halothane-treated versus untreated membranes. Membranes were treated as described in Materials and Methods. Pertussis toxin significantly increased adenylate cyclase activity versus control membranes in the absence of halothane after stimulation with isoprenaline ($p < 0.05$). *Numbers in the columns*, numbers of independent experiments performed with triplicate determinations.

adenylate cyclase. The activity of the catalyst was unaffected, as shown by experiments using forskolin. Thus, the sensitization of adenylate cyclase could be due to either an activation of $G_{s\alpha}$ or a functional inhibition of $G_{i\alpha}$.

Interaction of halothane with G proteins. To examine whether the inhibition of $G_{i\alpha}$ function provides an explanation for the effects of halothane, adenylate cyclase activity was determined in control and in pertussis toxin- plus NAD-treated membranes. Fig. 5 shows [^{32}P]ADP-ribosylation by pertussis toxin in control membranes and in membranes pretreated with pertussis toxin plus NAD. Pertussis toxin plus NAD treatment markedly attenuated [^{32}P]ADP-ribosylation by pertussis toxin of a 40-kDa protein, co-migrating with $G_{i\alpha}/G_{o\alpha}$ standard purified from bovine brain, present in human myocardial membranes and membranes from S49 wild-type and S49 *cyc*$^-$ cells. Thus, in membranes pretreated with pertussis toxin plus NAD a large portion of $G_{i\alpha}$ was covalently modified by the pretreatment. Pertussis toxin plus NAD treatment of myocardial membranes increased isoprenaline-, Gpp(NH)p-, and cholera toxin-stimulated adenylate cyclase activity (Fig. 6). Halothane augmented adenylate cyclase activity in control membranes but had no additional effect in pertussis toxin- plus NAD-treated membranes. These findings are compatible with pertussis toxin-sensitive inhibition of $G_{i\alpha}$ function. However, additional influences on $G_{s\alpha}$ could also exist. This appears to be relevant, because the effect of cholera toxin, which activates $G_{s\alpha}$ (20) by ADP-ribosylation (21) of an arginine residue at the GTPase site, was also enhanced (Fig. 6C). This finding could be compatible with an effect of halothane on cholera toxin action on $G_{s\alpha}$. In cholera toxin-treated membranes, hormonal $G_{i\alpha}$-mediated inhibition of adenylate cyclase is unchanged, as shown by the unchanged inhibitory effects of α_2-adrenergic receptor stimulation in platelet membranes (22) or somatostatin receptor stimulation in GH_3 cells (23). Therefore, the stimulatory effects of halothane could also be due to an impairment of $G_{i\alpha}$-mediated adenylate cyclase inhibition. The lack of halothane effects in pertussis toxin- plus NAD-treated membranes is in

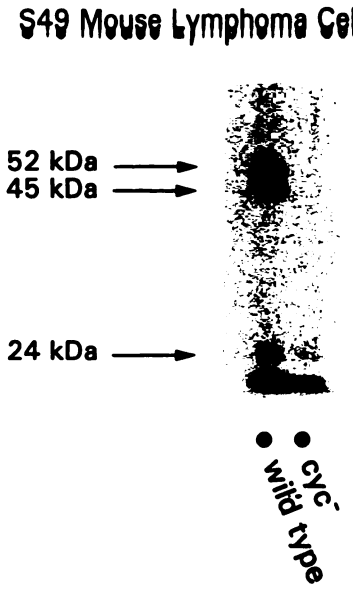

Fig. 8. [^{32}P]ADP-ribosylation by cholera toxin of $G_{s\alpha}$ (45 and 52 kDa) in S49 wild-type and S49 *cyc*$^-$ cell membranes. Membranes were treated with cholera toxin plus NAD and were separated by SDS-PAGE as described in Materials and Methods. In *cyc*$^-$ cell membranes the 45-kDa and 52-kDa cholera toxin substrates were lacking.

favor of the latter suggestion. Furthermore, we set out to determine the effects of halothane on $G_{i\alpha}$ and $G_{s\alpha}$ separately, by using S49 wild-type and $G_{s\alpha}$-deficient S49 *cyc*$^-$ cells. Fig. 7 shows the effects on S49 wild-type cell membranes. The increase by halothane of isoprenaline- and Gpp(NH)p-stimulated adenylate cyclase was observed only in control membranes and not in pertussis toxin- plus NAD-treated membranes. As in myocardial membranes (Fig. 4D), halothane did not augment adenylate cyclase stimulation by forskolin in S49 wild-type cell membranes, whether they had been treated with pertussis toxin plus NAD or not. Again, this finding is compatible with inhibition of $G_{i\alpha}$ function by halothane. If increased activity of $G_{s\alpha}$ plays a role in the effects of halothane, one would expect that the effects of halothane and pertussis toxin plus NAD treatment would be different in *cyc*$^-$ cell membranes, which genetically lack $G_{s\alpha}$ but clearly exhibit $G_{i\alpha}$-mediated inhibition of adenylate cyclase by hormones (24, 25) or guanine nucleotides (26, 27). Fig. 8 shows an autoradiogram of cholera toxin-catalyzed [^{32}P]ADP-ribosylation of S49 *cyc*$^-$ and wild-type cells, which were used in the following experiments. The 45-kDa and 52-kDa cholera toxin substrates, presumably $G_{s\alpha}$, could be identified in S49 wild-type membranes, whereas they were lacking in *cyc*$^-$ cells. As a consequence, isoprenaline and Gpp(NH)p did not stimulate adenylate cyclase activity in S49 *cyc*$^-$ cells (Fig. 9). The effect of forskolin was slightly less in S49 *cyc*$^-$ than in wild-type cell membranes. Fig. 10 summarizes the effects of halothane on basal adenylate cyclase activity from control and pertussis toxin- plus NAD-treated membranes prepared from human myocardium (Fig. 10A), S49 wild-type cells (Fig. 10B), S49 *cyc*$^-$ cells (Fig. 10C), and S49 *cyc*$^-$ cells reconstituted with $rG_{s\alpha}$ from *E. coli* (Fig. 10D). $rG_{s\alpha}$ were reconstituted in a final concentration of 0.5 μg/μl of *E. coli* lysates. Halothane increased basal adenylate cyclase activity to a similar extent (i.e., 30–50%) under all conditions studied. Under all conditions, the stimulatory effects of halothane did not occur when the activity was already increased by pretreatment of the membranes with pertussis toxin plus NAD. Thus,

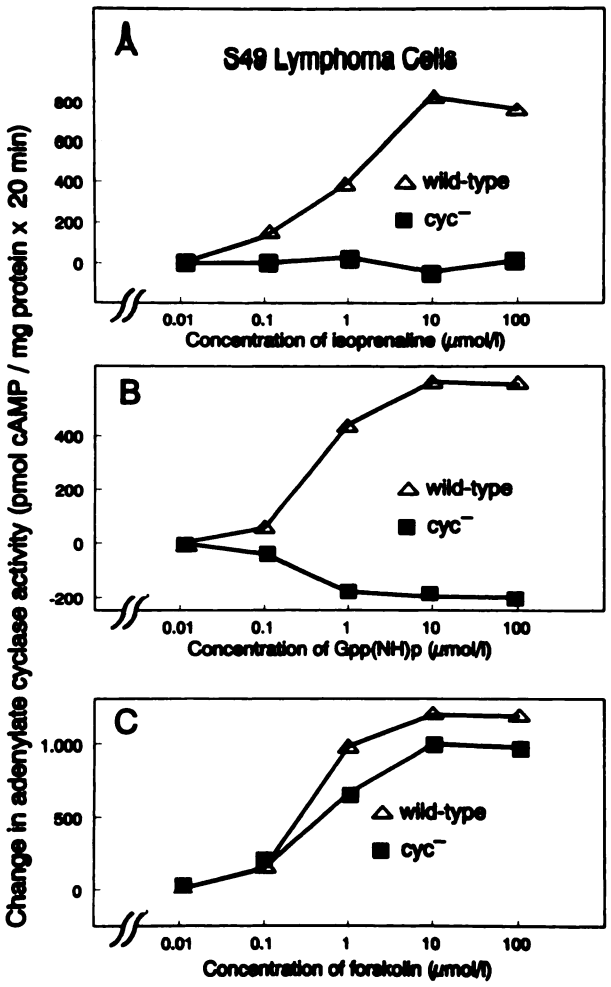

Fig. 9. Concentration-response curves for the effects of isoprenaline (0.01–100 μM) (A), Gpp(NH)p (0.01–100 μM) (B), and forskolin (0.01–100 μM) (C) in S49 wild-type and S49 *cyc*$^-$ cell membranes. *Ordinates*, change in adenylate cyclase activity. *Abscissae*, concentrations of studied agents.

even in $G_{s\alpha}$-deficient S49 *cyc*$^-$ cells, halothane produced a pertussis toxin-sensitive stimulation of adenylate cyclase activity. Reconstitution of $G_{s\alpha}$-deficient S49 *cyc*$^-$ cells with $rG_{s\alpha}$ concentration-dependently restored the effect of isoprenaline on adenylate cyclase (data not shown). Halothane in these reconstituted membranes enhanced the restored isoprenaline- and Gpp(NH)p-stimulated cyclase in a pertussis toxin-sensitive way (data not shown), similar to that in S49 wild-type or myocardial membranes. These experiments show that the pertussis toxin-sensitive halothane effects are not influenced by the presence of native or reconstituted $rG_{s\alpha}$. A more direct approach used to study impairment of $G_{i\alpha}$ function by halothane was measurement of the GTP- or Gpp(NH)p-induced inhibition of adenylate cyclase in S49 *cyc*$^-$ membranes. Fig. 11A summarizes the effect of halothane on adenylate cyclase in the presence of forskolin. GTP at 100 μM inhibited forskolin-stimulated adenylate cyclase activity by 32% in control membranes and by 15% in halothane-treated membranes. Inhibition by GTP was significantly different in control and halothane-treated membranes. Addition of somatostatin further inhibited adenylate cyclase by 10% in control and halothane-treated membranes. The effects of somatostatin were similar under both conditions. Similar results were obtained when the effect

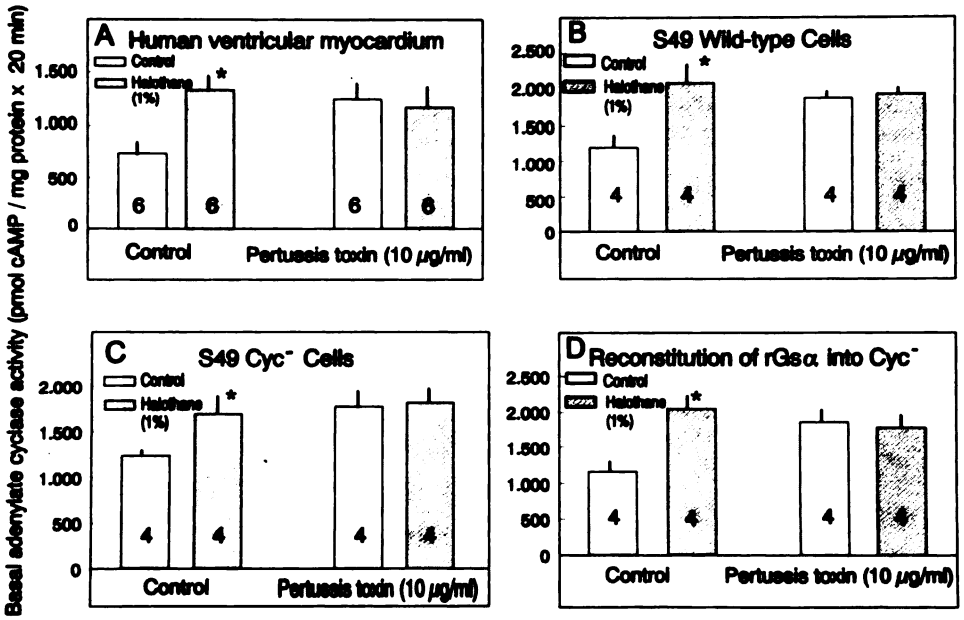

Fig. 10. Basal adenylate cyclase activity in control (*left*) and pertussis toxin- plus NAD-treated (*right*) membranes from human ventricular heart muscle (A), S49 wild-type cell membranes (B), S49 *cyc*⁻ cell membranes (C), and S49 *cyc*⁻ cell membranes reconstituted with $rG_{s\alpha}$ (D), in the absence (□) and presence (▨) of halothane. Pertussis toxin significantly increased adenylate cyclase activity versus control membranes in the absence of halothane. *Numbers in the columns*, numbers of independent experiments performed with triplicate determinations. *Ordinates*, basal adenylate cyclase. *Abscissae*, studied conditions. Membranes were treated as described in Materials and Methods.

of Gpp(NH)p was investigated. Fig. 11B shows concentration-response curves for the effects of halothane on Gpp(NH)p- and Gpp(NH)p- plus somatostatin-inhibited adenylate cyclase. Halothane attenuated Gpp(NH)p- and Gpp(NH)p- plus somatostatin-induced inhibition of adenylate cyclase. However, the additional effect of somatostatin was maintained in control and halothane-treated membranes.

Taken together, these data indicate that halothane similarly increased basal adenylate cyclase activity in human myocardial, S49 *cyc*⁻, and S49 wild-type cell membranes, as well as in S49 *cyc*⁻ cell membranes reconstituted with $rG_{s\alpha}$. In addition, stimulation by isoprenaline and Gpp(NH)p, but not by forskolin, was enhanced by halothane. The effects were similar in all membranes studied and were completely sensitive to pertussis toxin plus NAD treatment. Thus, the findings presented point to inactivation of $G_{i\alpha}$ function as the mechanism for the stimulatory effects of halothane on adenylate cyclase activity in human myocardial membranes and S49 mouse lymphoma cells.

Effects of halothane on M-cholinoceptor-G_i interaction. To address the question of whether the influence of halothane interferes with M-cholinoceptor coupling in the myocardium, the effects of the hydrocarbon anesthetic on carbachol-stimulated [^{35}S]GTPγS binding were studied. This experimental approach was necessary, because we were not able to measure a reliable inhibition of adenylate cyclase (<25%) in human myocardial membranes. Carbachol-stimulated [^{35}S]GTPγS binding was critically dependent on the presence of GDP in the assay medium. In the presence of 0.3 µM GDP, carbachol stimulated [^{35}S]GTPγS binding by about 50%. Halothane had no influence on carbachol-activated [^{35}S]GTPγS binding to myocardial membranes (Fig. 12).

Discussion

Halothane produces negative inotropic effects on isolated cardiac preparations *in vitro* and in patients *in vivo* (28). In addition, sensitization of the myocardium towards exogenous catecholamines has been described (8–10). In patients anesthetized with hydrocarbon anesthetics, the ability of catecholamines to induce cardiac arrhythmias was observed to be facilitated, with halothane being the most potent agent (10). This catecholamine-sensitizing effect was observed in dogs treated with halothane even at subanesthetic concentrations (11). Although this phenomenon is often described in textbooks, the underlying mechanisms are not known. In this study, the positive inotropic effect of isoprenaline on isolated cardiac preparations in the absence and presence of halothane was investigated as a model to study the catecholamine-sensitizing effect of halothane in human myocardium. The positive inotropic effect of isoprenaline was increased by halothane, whereas the positive inotropic effect of an increase of the extracellular Ca^{2+} concentration was unchanged. The potentiation of the isoprenaline effect by halothane was more pronounced in failing, compared with nonfailing, myocardium. Because isoprenaline is not subject to uptake into presynaptic vesicles as a mechanism of inactivation (29), the underlying mechanism should be located at the G protein-regulated adenylate cyclase of the myocardial cell. Therefore, we next set out to characterize the subcellular mechanism of the catecholamine-sensitizing effects on adenylate cyclase activity.

On the cellular level, cardiac adenylate cyclase is dually regulated by stimulatory and inhibitory receptors (30). The formation of cAMP from ATP by the catalyst is stimulated by cardiac β_1- and β_2-adrenoceptors via a heterotrimeric stimulatory G protein ($\alpha_s\beta\gamma$). The catalyst is under the inhibitory control of inhibitory G proteins ($\alpha_i\beta\gamma$), which couple A_1 adenosine receptors (31) and M_2 cholinoceptors (32). The α subunits of this family of G proteins are subject to covalent modification by the ADP-ribosyltransferase activity of pertussis toxin, which modifies a cysteine residue at the fourth position from the carboxyl terminus (30). This covalent modification of the α subunits leads to functional inactivation of $G_{i\alpha}$, resulting in lost inhibition of basal and receptor-modulated adenylate cyclase activity (33). Although adenylate cyclase desensitization in the failing human heart due to down-regulation of β-adrenoceptors (34) and an increase of $G_{i\alpha}$ (35) is well characterized, nothing is known about the mechanisms of sensitization, e.g., after application of halothane. From the complex regulation of cardiac adenylate cyclase activity, it is evident that mechanisms

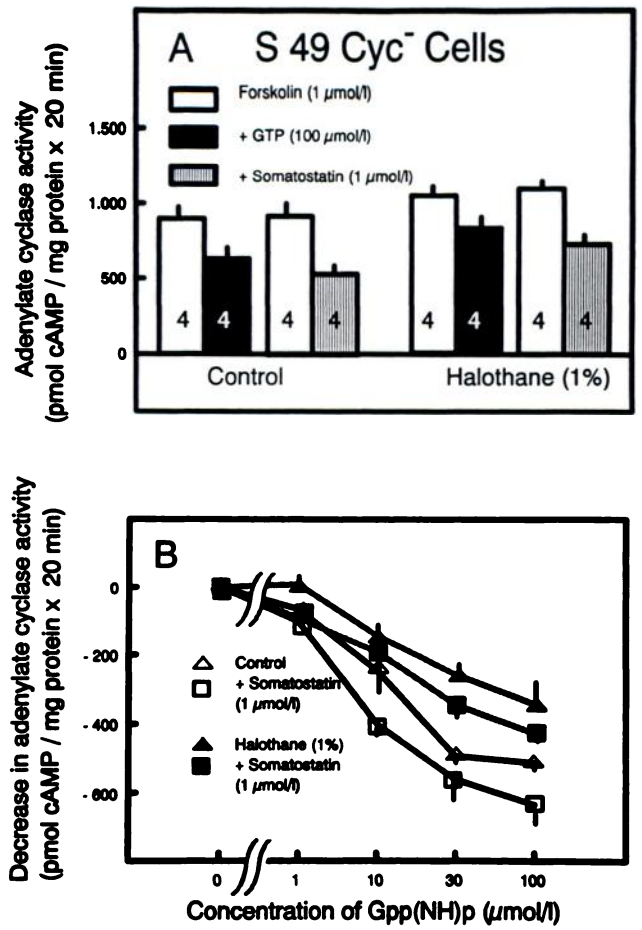

Fig. 11. Effect of halothane on adenylate cyclase in forskolin-, forskolin- plus GTP-, and forskolin- plus GTP- plus somatostatin-treated cells (A) and on concentration-dependent inhibition of forskolin-stimulated adenylate cyclase activity by Gpp(NH)p (1–100 μM), alone and in the presence of somatostatin (B). *Ordinates*, increase (A) or decrease (B) of adenylate cyclase activity in S49 *cyc*⁻ membranes. *Abscissae*, studied conditions (A) or concentrations of Gpp(NH)p (B). Basal adenylate cyclase activities were 405 ± 25 pmol of cAMP/mg of protein × 20 min (n = 20) (plus halothane, 492 ± 41 pmol of cAMP/mg of protein × 20 min, n = 20). Forskolin stimulated adenylate cyclase activity by 1090 ± 98 pmol of cAMP/mg of protein × 20 min (n = 20) (plus halothane, 1074 ± 10 pmol of cAMP/mg of protein × 20 min, n = 20). *Numbers in the columns*, numbers of independent experiments performed with triplicate determinations.

of sensitization could involve the catalyst, G proteins like $G_{s\alpha}$ or $G_{i\alpha}$, and receptors.

In experiments on isolated membranes, not only the effects of isoprenaline but also those of the poorly hydrolyzable guanine nucleotide Gpp(NH)p and NaF were enhanced by halothane. In uterine membranes, halothane augmented guanine nucleotide-stimulated adenylate cyclase activity (36). From these observations it is evident that halothane is able to sensitize adenylate cyclase independently of β-adrenoceptors, either by an increase of the catalyst or $G_{s\alpha}$ activity or by impairment of $G_{i\alpha}$ function. Effects of halothane on β-adrenoceptor-$G_{s\alpha}$ coupling cannot be completely ruled out by these experiments. However, halothane inhibited high affinity agonist binding to β-adrenoceptors (37). This finding suggests an uncoupling rather than a facilitated coupling of β-adrenoceptors to adenylate cyclase and does not explain the enhanced isoprenaline-stimulated adenylate cyclase activity.

The diterpene derivate forskolin is known to stimulate the adenylate cyclase catalyst (18). The unchanged effect of forskolin in the presence of halothane points to unchanged activity of the catalyst. To study whether halothane exerts effects on the inhibitory G protein in the heart, the effects of the anesthetic on isoprenaline-, Gpp(NH)p-, and cholera toxin-stimulated adenylate cyclase were studied in native and pertussis toxin- plus NAD-treated membranes. In human failing heart muscle the expression of $G_{i\alpha}$ is increased, and $G_{i\alpha}$ has been suggested to be involved in the reduced adenylate cyclase activity observed with this condition (34, 35). Treatment of cardiac membranes of nonfailing and failing myocardium with pertussis toxin restored adenylate cyclase activity (35). The authors of the latter study suggested that $G_{i\alpha}$ was completely inactivated by pertussis toxin treatment (35) and that studies on pertussis toxin-treated membranes provide a model to study the function of $G_{i\alpha}$. The same technique was used herein to inactivate $G_{i\alpha}$. In pertussis toxin-treated myocardial membranes, adenylate cyclase activity was enhanced but the stimulatory effect of halothane was abolished. These observations strongly favor the conclusion that halothane inhibits $G_{i\alpha}$ function. Because halothane and pertussis toxin increased adenylate cyclase activity to similar extents, it is possible that halothane acts by withdrawing tonic inhibitory effects of $G_{i\alpha}$ on the enzyme. This suggestion is further supported by the functional data. Halothane increased the positive inotropic effect of isoprenaline more in failing myocardium with increased $G_{i\alpha}$, than in nonfailing myocardium.

Although the experiments with pertussis toxin provide evidence for an impaired $G_{i\alpha}$ function produced by halothane, additional effects on $G_{s\alpha}$ cannot be ruled out with certainty. Therefore, the same experiments were performed in S49 *cyc*⁻ and wild-type cell membranes. In *cyc*⁻ and wild-type cell membranes, halothane increased basal adenylate cyclase activity similarly as in myocardial membranes. Because *cyc*⁻ cells genetically lack $G_{s\alpha}$ and the stimulatory actions of halothane were again abolished by pertussis toxin plus NAD treatment, these experiments provide additional evidence that inactivation of $G_{i\alpha}$ rather than increased activity of $G_{s\alpha}$ is the underlying mechanism of action. In agreement with this notion is the finding that the sensitizing effects of halothane on stimulated adenylate cyclase activity were similar in human myocardial, S49 wild-type, and *cyc*⁻ cell membranes reconstituted with exogenous $rG_{s\alpha}$.

When $G_{i\alpha}$ is inactivated by halothane, one would expect that receptor coupling to $G_{i\alpha}$ would be also impaired. However, halothane had no effect on carbachol-stimulated [^{35}S]GTPγS binding. This observation is in accordance with a previously published study that showed that the direct negative inotropic effect in human atrial myocardium and the antiadrenergic effect in human ventricular myocardium of carbachol is not influenced by halothane (37). Therefore, halothane appears to inhibit $G_{i\alpha}$ effects on adenylate cyclase independently of receptor coupling. In agreement, halothane attenuated GTP- and Gpp(NH)p-mediated inhibition of adenylate cyclase in S49 *cyc*⁻ cell membranes, whereas the inhibitory effect of somatostatin was unchanged. In this respect, it is interesting that in failing human heart the effects of M-cholinoceptor and A_1 adenosine receptor agonists are unchanged (38, 39), although $G_{i\alpha}$ is increased (35, 38, 39). The discrepancy regarding the unchanged coupling of inhibitory receptors in the presence of an altered $G_{i\alpha}$ content or function could be explained by the receptor-G

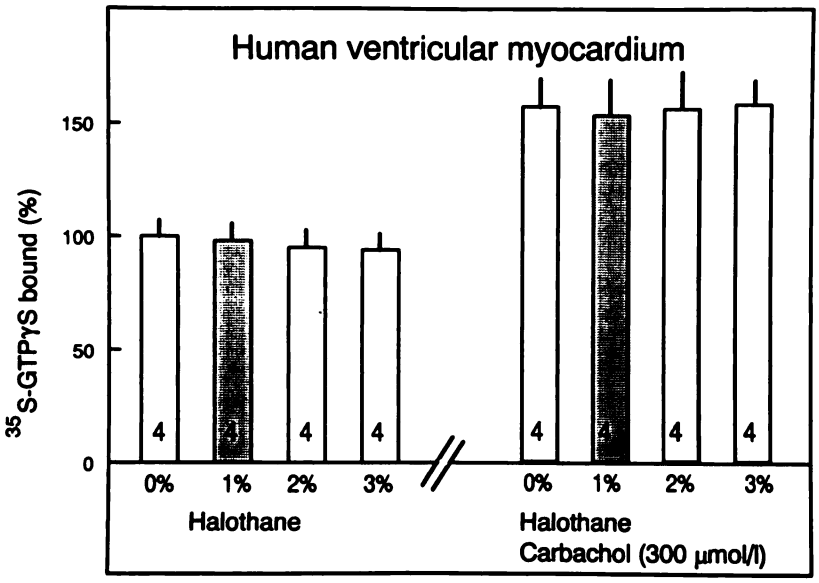

Fig. 12. Basal and carbachol-stimulated [^{35}S]GTPγS binding, alone and in the presence of halothane, in human ventricular membranes. The experiments were performed in the presence of 0.3 µM GDP. Absolute values for [^{35}S]GTPγS binding sites were 30 pmol/mg of protein, as judged from Scatchard analysis of GTPγS competition experiments without GDP (data not shown). *Ordinate*, [^{35}S]GTPγS binding as a percentage of the value for the control condition in the absence of carbogen or halothane treatment. *Abscissa*, studied conditions. The values represent the mean of four independent experiments performed with triplicate determinations.

protein stoichiometry. $G_{i\alpha}$ proteins are present in an excess of about 1000, compared with the receptor number, in human heart. Thus, inactivation of a major part, but not all, of the $G_{i\alpha}$ proteins by halothane could still leave some $G_{i\alpha}$ proteins functionally unchanged, allowing maximal coupling of inhibitory M-cholinoceptors in myocardium or somatostatin receptors in S49 *cyc*$^-$ cell membranes. In this investigation, we have studied similar failing human hearts with increased $G_{i\alpha}$ levels. The insensitivity of receptor-G protein coupling to variations in the function or amount of G proteins in human heart is an important difference, compared with other tissues such as brain, where an interruption of receptor-G protein coupling was observed previously (1, 3–7).

The results of this study highlight conceptual issues regarding the possibility of pharmacologically influencing G protein activity and add a new class of agents, i.e., hydrocarbon anesthetics, as potential G protein antagonists. The described effects of these compounds could play a crucial role in their narcotic effects. In addition, antagonism of $G_{i\alpha}$ function might explain the sensitization of the myocardium towards exogenous catecholamines by halothane. The data presented herein demonstrate for the first time that inactivation of $G_{i\alpha}$ leads to an increase of positive inotropic responses in human heart. Finally, the present results indicate that pharmacological inhibition of $G_{i\alpha}$ function without effects on $G_{s\alpha}$ is feasible. This latter observation sets the stage for the development of experimental therapeutic agents to inactivate $G_{i\alpha}$, e.g., in failing human heart, to reverse heterologous adenylate cyclase desensitization, and to restore cAMP formation and inotropic responsiveness in this condition.

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References

1. Aronstam, R. S., and R. L. Dennison. Anesthetic effects on muscarinic signal transduction. *Int. Anesthesiol. Clin.* **27**:265–272 (1989).
2. Franks, N. P., and W. R. Lieb. Do general anaesthetics act by competitive binding to specific receptors? *Nature (Lond.)* **310**:599–601 (1984).
3. Fraser, M. D., S. R. W. Louro, L. I. Horvath, K. W. Miller, and A. Watts. A study of the effect of general anesthetics on lipid-protein interactions in acetylcholine receptor enriched membranes from *Torpedo nobiliana* using nitroxide spin labels. *Biochemistry* **29**:2664–2669 (1990).
4. Anthony, B. L., R. L. Dennison, and R. S. Aronstam. Disruption of muscarine receptor-G protein coupling is a general property of liquid volatile anesthetics. *Neurosci. Lett.* **99**:191–196 (1989).
5. Aronstam, R. S., B. L. Anthony, and R. L. Dennison. Halothane effects on muscarinic acetylcholine receptor complexes in rat brain. *Biochem. Pharmacol.* **35**:667–672 (1986).
6. Martin, D. C., R. L. Dennison, R. P. S. Introna, and R. S. Aronstam. Influence of halothane on the interactions of serotonin$_{1A}$ and adenosine A_1 receptors with G proteins in rat brain membranes. *Biochem. Pharmacol.* **42**:1313–1316 (1991).
7. Baumgartner, M. K., R. L. Dennison, T. K. Narayanan, and R. S. Aronstam. Halothane disruption of α_2-adrenergic receptor-mediated inhibition of adenylate cyclase and receptor G protein coupling in rat brain. *Biochem. Pharmacol.* **39**:223–225 (1990).
8. Reynolds, A. K. On the mechanism of myocardial sensitization to catecholamines by hydrocarbon anesthetics. *Can. J. Physiol. Pharmacol.* **62**:183–198 (1984).
9. Bosnjak, Z. J., and L. A. Turner. Halothane, catecholamines, and cardiac conduction: anything new? *Anesth. Analg.* **72**:1–4 (1991).
10. Johnston, R. R., E. I. Eger, and C. Wilson. A comparative interaction of epinephrine with enflurane and halothane in man. *Anesth. Analg.* **55**:709–712 (1976).
11. Hayashi, Y., K. Sumikawa, A. Yamatodani, T. Kamibayashi, M. Kuro, and I. Yoshiya. Myocardial epinephrine sensitization with subanesthesic concentrations of halothane in dogs. *Anesthesiology* **74**:134–137 (1991).
12. Gierschik, P., M. Steisslinger, D. Sidiropoulos, E. Herrmann, and K. H. Jakobs. Dual Mg^{2+} control of formyl peptide receptor-G protein interaction: evidence that low-agonist-affinity receptor interacts with and activates the G protein. *Eur. J. Biochem.* **183**:97–105 (1989).
13. Graziano, M. P., P. J. Casey, and A. G. Gilman. Expression of cDNAs for G proteins in *Escherichia coli*. *J. Biol. Chem.* **262**:11375–11381 (1987).
14. Lin, K., and S. Cheng. An efficient method to purify active eukaryotic proteins from the inclusion bodies in *Escherichia coli*. *Biotechniques* **11**:748–751 (1991).
15. Hiroshi, I., and A. G. Gilman. Expression and analysis of $G_{s\alpha}$ mutants with decreased ability to activated adenylyl cyclase. *J. Biol. Chem.* **266**:16226–16231 (1991).
16. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurements with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
17. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)* **227**:680–685 (1970).
18. Böhm, M., K. Larisch, and E. Erdmann. Quantification of $G_{i\alpha}$ by a novel radioimmunoassay in cardiomyopathic human hearts. *Naunyn Schmiedebergs Arch. Pharmacol.* **343**(suppl.):R54 (1991).
19. Seamon, K. B., and J. W. Daly. Forskolin: its biological and chemical properties. *Adv. Cyclic Nucleotide Protein Phosphorylation Res.* **20**:1–150 (1989).
20. Cassel, D., and Z. Selinger. Mechanism of adenylate cyclase activation by cholera toxin: inhibition of GTP hydrolysis at the regulatory site. *Proc. Natl. Acad. Sci. USA* **74**:3307–3311 (1977).
21. Cassel, D., and T. Pfeuffer. Mechanism of cholera toxin action: covalent

modification of the guanyl nucleotide-binding protein of the adenylate cyclase system. *Proc. Natl. Acad. Sci. USA* **75**:2669–2673 (1978).
22. Gierschik, P., and K. H. Jakobs. Mechanisms for inhibition of adenylate cyclase by *alpha*-2 adrenergic receptors, in *The Alpha-2 Adrenergic Receptors* (L. E. Limbird, ed.). Humana Press, Clifton, NJ, 75–113 (1988).
23. Toro, M. J., E. Montoya, and L. Birnbaumer. Inhibitory regulation of adenylyl cyclases: evidence inconsistent with β-complexes of G_i proteins mediating hormonal effects by interfering with activation of G_s. *Mol. Endocrinol.* **1**:669–677 (1987).
24. Jakobs, K. H., and G. Schultz. Occurrence of a hormone-sensitive inhibitory coupling component of the adenylate cyclase in S49 lymphoma *cyc*$^-$ variants. *Proc. Natl. Acad. Sci. USA* **80**:3899–3902 (1983).
25. Jakobs, K. H., K. Aktories, and G. Schultz. A nucleotide regulatory site for somatostatin inhibition of adenylate cyclase in S49 lymphoma cells. *Nature (Lond.)* **303**:177–178 (1983).
26. Hildebrandt, J. D., R. D. Sekura, J. Codina, R. Iyengar, C. R. Manclark, and L. Birnbaumer. Stimulation and inhibition of adenyl cyclases mediated by distinct regulatory proteins. *Nature (Lond.)* **302**:706–709 (1983).
27. Hildebrandt, J. D., J. Hanoune, and L. Birnbaumer. Guanine nucleotide inhibition of *cyc*$^-$ S49 mouse lymphoma cell membrane adenylyl cyclase. *J. Biol. Chem.* **257**:14723–14725 (1982).
28. Lynch, C. Differential depression of myocardial contractility by halothane and isoflurane *in vitro*. *Anesthesiology* **64**:620–631 (1986).
29. Callingham, B. A., and A. S. V. Burgen. The uptake of isoprenaline and noradrenaline by the perfused rat heart. *Mol. Pharmacol.* **2**:37–42 (1966).
30. Gilman, A. G. G proteins: transducers of receptor-generated signals. *Annu. Rev. Biochem.* **56**:615–649 (1987).
31. Böhm, M., R. Brückner, J. Neumann, W. Schmitz, H. Scholz, and J. Starbatty. Role of guanine nucleotide binding protein in the regulation by adenosine of cardiac potassium conductance and force of contraction. *Naunyn Schmiedebergs Arch. Pharmacol.* **332**:403–405 (1986).
32. Sorota, S., Y. Tsuji, T. Tajima, and A. J. Pappano. Pertussis toxin treatment blocks hyperpolarization by muscarine agonists in chick atrium. *Circ. Res.* **57**:748–758 (1985).
33. Hazeki, O., and M. Ui. 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).
34. Bristow, M. R., R. Ginsburg, W. Minobe, R. S. Cubicciotti, W. S. Sageman, K. Lurie, M. E. Billingham, D. C. Harrison, and E. B. Stinson. Decreased catecholamine sensitivity and β-adrenergic-receptor density in failing human hearts. *N. Engl. J. Med.* **307**:205–211 (1982).
35. Feldman, A. M., A. E. Cates, W. B. Veazey, R. E. Hershberger, M. R. Bristow, K. L. Baughman, W. A. Baumgartner, and C. Van Dop. Increase of the 40,000-mol wt pertussis toxin substrate (G protein) in failing human heart. *J. Clin. Invest.* **82**:189–197 (1988).
36. Triner, L., Y. Vulliemoz, and M. Verosky. The action of halothane on adenylate cyclase. *Mol. Pharmacol.* **13**:976–979 (1977).
37. Böhm, M., U. Schmidt, R. H. G. Schwinger, S. Böhm, and E. Erdmann. Effects of halothane on β-adrenoceptors and M-cholinoceptors in human myocardium: radioligand binding and functional studies. *J. Cardiovasc. Pharmacol.* **21**:296–304 (1993).
38. Böhm, M., P. Gierschik, K. H. Jakobs, B. Pieske, P. Schnabel, M. Ungerer, and E. Erdmann. Increase of $G_{i\alpha}$ in human hearts with dilated but not ischemic cardiomyopathy. *Circulation* **82**:1249–1265 (1990).
39. Hershberger, R. E., A. M. Feldman, and M. R. Bristow. A_1-adenosine receptor inhibition of adenylate cyclase in failing and nonfailing human ventricular myocardium. *Circulation* **83**:1342–1351 (1991).

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