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Halothane, But Not Isoflurane, Impairs the β-adrenergic Responsiveness in Rat Myocardium

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Background: **The aim of this study was to identify the mechanisms by which halothane and isoflurane change the myocardial β-adrenergic signal transduction pathway.**

Methods: **The authors investigated the influence of volatile anesthetics on the isometric force of contraction of rat papillary muscles. Concentration–response curves for isoproterenol and epinephrine were studied under control conditions and in the presence of halothane or isoflurane. In radioligand receptor-binding studies, the β-adrenoceptor affinities for isoproterenol and epinephrine were investigated with and without guanosine triphosphate. In addition, the isoproterenol-induced cyclic adenosine monophosphate accumulations in viable cardiomyocytes in the absence and in the presence of halothane were determined by radioimmunoassays.**

Results: **The half-maximal positive inotropic effect of isoproterenol was reached at a half-maximal effective concentration (EC_{50} value) of 68 nM (33–141 nM; n = 10). A minimum alveolar concentration of 1.3 halothane reduced the positive inotropic potency of isoproterenol (EC_{50} = 158 nM [118–214 nM; n = 10; $P < 0.01$ *vs.* control]), whereas isoflurane did not changed it. This observation held true when the force of contraction was stimulated with epinephrine. Halothane (1.3 minimum alveolar concentration) depressed β-adrenoceptor high-affinity binding and β-adrenoceptor agonist affinity in radioligand binding assays, an effect not seen with isoflurane. Halothane shifted the intracellular cyclic adenosine monophosphate response curve of isoproterenol to the right.**

Conclusion: **Halothane, but not isoflurane, impairs the β-adrenergic responsiveness in rat myocardium by reducing the agonist affinity of the β-adrenoceptors. (Key words: β-adrenoceptor; contraction experiments; radioimmunoassay; radioligand binding; volatile anesthetics.)**

THE cardiodepressant effect of volatile anesthetics is presumed to be a result of several mechanisms.[1–4] Volatile anesthetics attenuate the slow inward Ca^{2+} current into cardiomyocytes[5] and deplete Ca^{2+} stores in the sarcoplasmic reticulum.[6,7] In addition, they seem to interact directly with the myofibrils.[8,9] Besides these effects, volatile anesthetics alter the myocardial response to β-adrenergic stimulation. The potency of various catecholamines to increase force of contraction of isolated muscle preparations is reduced in the presence of halothane in the rat,[10] guinea pig,[11] and in humans.[4] On the other hand, halothane has been reported to facilitate catecholamine-induced arrhythmias in laboratory animals[12] and in anesthetized patients.[13] The discrepancy between these different interactions with catecholamines raises questions about the effect of volatile anesthetics on the transsarcolemmal β-adrenergic signal transduction in the heart. Several groups investigated the influence of halothane on the binding properties of β-adrenoceptors. Bernstein *et al.*[14] and Marty *et al.*[15] showed only minor changes in β-adrenoceptor density in canine myocardium or lymphocytes, respectively, determined in the presence or absence of halothane in the binding assays. Only a few studies focused on the effects of volatile anesthetics on the agonist affinity of β-adrenoceptors[16] or on the agonist-induced intracellular cyclic adenosine monophosphate (cAMP) accumulation.[17]

Halothane and isoflurane interact with the effect of catecholamines on the heart in different ways. Isoflurane produces less potentiation of the proarrhythmo-

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genic effect and a smaller reduction of the positive inotropic potency of catecholamines compared with halothane.[4,13] Therefore, we compared the influence of halothane and isoflurane on the β-adrenergic signal transmission in rat myocardium. We studied the positive inotropic effect of different catecholamines on left ventricular papillary muscles in the presence of both anesthetics. We used isolated adult rat cardiomyocytes in radioligand binding studies to determine the effects of anesthetics on the agonist affinity of β-adrenoceptors and on isoproterenol-induced cAMP accumulation in cardiomyocytes.

Materials and Methods

The study was performed in accordance with the guidelines of the institutional review committee on research and the declaration of Helsinki.

Force of Contraction of Rat Papillary Muscles

Female Sprague-Dawley rats (weight, 160–180 g) were killed by cervical dislocation, the hearts were quickly excised and placed in a bathing solution consisting of 1.8 mM $CaCl_2$, 120 mM NaCl, 1.04 mM $MgCl_2$, 5.35 mM KCl, 22.6 mM $NaHCO_3$, 0.42 mM NaH_2PO_4, 5.05 mM glucose, 0.28 mM ascorbic acid, 0.05 mM Na_2EDTA, at *p*H 7.4, gassed with 5% carbon dioxide and 95% oxygen (carbogen), and kept at 37°C. Papillary muscles (diameter <0.7 mm) were prepared from left ventricles under stereomicroscopic control. The force of contraction was measured with inductive force transducers (W. Fleck, Mainz, Germany) connected to a recorder (Hellige, Freiburg, Germany) and determined under isometric conditions as described previously.[18] The muscles were driven electrically at 1 Hz with rectangular impulses (lasting 5 ms) of a voltage 5–10% above threshold. The force of contraction of each muscle was allowed to reach steady state. During this time (10–90 min), the bathing solution was changed every 15 min, and the resting force (about 10 mN) was maintained until the force of contraction was maximal (*i.e.*, until the sarcomeric length was optimal). This length was kept constant throughout the experiment.

We studied the negative inotropic effects of halothane and isoflurane and the positive inotropic effects of isoproterenol and epinephrine in the presence of either anesthetic. The anesthetics were added to the carbogen using vaporizers (we used Vapor 19 from Dräger, Lübeck, Germany for halothane or Isotec 3 from Ohmeda, Steeton, UK for isoflurane) and bubbled into the organ baths. Gas chromatography measurements revealed that equilibration of the anesthetics with the bathing solution was complete within 5 min. Concentration–response curves for isoproterenol (0.1 nM to 10 μM) or epinephrine (10 nM to 100 μM) were performed by adding the drugs cumulatively to the organ chambers after the steady state condition of the previous effects was achieved for at least 5 min. To determine whether the reduction of the positive inotropic potency of catecholamines in the presence of halothane is the result of an alteration of muscarinic effects on adenylyl cyclase observed in previous investigations,[19] we also studied the effects of carbachol (10 nM to 10 μM) on papillary muscles that were prestimulated with isoproterenol (0.3 μM).

Isolation of Cardiomyocytes

Calcium-tolerant, rod-shaped cardiomyocytes from rats were obtained by a method described previously.[20] Briefly, female Sprague-Dawley rats (weight, 160–180 g) were killed by cervical dislocation, and the hearts were quickly excised and perfused under constant pressure (70 cm H_2O) at 37°C using the Langendorff technique with a solution (A) containing 138 mM NaCl, 5.9 mM KCl, 0.5 mM KH_2PO_4, 2.5 mM Na_2PO_4, 2 mM $MgSO_4$, 10 mM Hepes, 5.5 mM glucose, 2 mM pyruvate, at *p*H 7.55. After 5 min, 7 mg/ml bovine serum albumin, 15 mM 2,3-butanedionemonoxime, and 1 mg/ml collagenase were added to the solution. The Ca^{2+} concentration was increased to 100 μM after 10 min and to 200 μM after 15 min (solution B). After 30 min of perfusion, the hearts were teased into pieces with tweezers and shaken in solution B but without collagenase for 10 min. Subsequently, the Ca^{2+} concentration was increased in five steps to 1.8 mM. The cell suspension was filtered using a 200 × 200 μm mesh and centrifuged for 45 s at 18*g*. After resuspension of the pellet in the same medium, the cells were allowed to settle for 2.5 min. The pellet was resuspended in oxygenated incubation buffer (medium 199 also containing 20 mg/ml bovine serum albumin at *p*H 7.55) or in binding buffer (solution C: 10 mM $MgCl_2$, 50 mM Tris-HCl, at *p*H 7.4). The dissociation procedure provided many (6–10 million) single cardiac cells of which 80–90% were rod-shaped and had distinct striation patterns, well-defined cell borders, and no bubbles on the cell surface. These cells were considered to be viable.[21]

Radioligand Binding Experiments

After freshly isolated cardiomyocytes had been resuspended in buffer C they were centrifuged at 50,000*g* and 4°C for 20 min. The resulting pellet of cell fragments was resuspended in 15 ml of the same buffer, quickly frozen in liquid nitrogen, and stored at −80°C until use. All the following steps were performed at 4°C. The cell preparations were washed four times by the same centrifugation procedure in buffer C to remove guanosine triphosphate (GTP). The successful removal of GTP allowed us to distinguish the high-affinity from the low-affinity binding state of the β-adrenoceptors,[22] as described in the Results section. The cell preparation was gassed for 15 min with anesthetic in nitrogen or with pure nitrogen for control to avoid oxidative alterations of membrane lipids. The radioligand binding experiments were performed in an anesthetic-nitrogen atmosphere or in pure nitrogen, respectively. An aliquot of the preparation containing 60 μg protein in 150 μl buffer was incubated with the radiolabeled β-adrenoceptor antagonist ^{125}I-Iodocyanopindolol (ICYP) in a final volume of 250 μl for 60 min at 37°C according to Brodde *et al.*[23] ^{125}I-Iodocyanopindolol binding that was not displaced by 1 μM of the β-adrenoceptor antagonist CGP 12177 (4-(3-teriarybutylamino-2-hydroxypropoxyl)-benzimidazole-2-one) was considered nonspecific. Specific binding of ICYP was defined as the total bound radioactivity minus nonspecific binding and was about 85% of total binding at 10 pM ICYP. To evaluate the β-adrenoceptor density in cardiomyocyte preparations, saturation binding experiments were performed in which specifically bound ICYP was determined at seven different concentrations of ICYP ranging from 5-300 pM. The incubation was terminated by adding 4 ml ice-cold buffer C to the entire incubation mixture, followed by rapid filtration through Whatman GF/C glass fiber filters. Each filter was washed with an additional 8 ml buffer. The radioactivity retained in the filters was determined in a gamma counter (Beckmann, Munich, Germany) at an efficiency of 80%.

To determine the potency of isoproterenol or epinephrine to inhibit binding (*i.e.*, to determine the affinity of the agonists to the β-adrenoceptors), 22 different concentrations of isoproterenol (20 pM to 500 μM) or epinephrine (0.2 nM to 3 mM) were added to the assays. In one half of the assays, GTP was replaced to the final concentration of 100 μM. The subsequent steps of the binding experiments were performed as described above. The protein content was measured according to the method of Bradford[24] using bovine serum albumin as standard.

Determination of Cyclic Adenosine Monophosphate Levels in Viable Rat Cardiomyocytes Exposed to Halothane

Freshly isolated cardiomyocytes were resuspended to a density of approximately 35,000 cells/ml in medium 199 containing 0.5 mM of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine. Cells were incubated at 37°C in air or halothane vapor (3 vol%), respectively. As determined by gas chromatography, the halothane concentration was equilibrated in the medium within 30 min. After this equilibration period, concentration-response curves were performed by exposing the cells to 10 different concentrations of isoproterenol (0.1 nM to 0.1 mM) for 10 min. The incubation was terminated by adding 100 μl 1 N HCl to 1 ml cell suspension, respectively, rough vortexing, and immersing the suspension in liquid nitrogen. These preparations were stored at −80°C until cAMP determination. After centrifugation (13,000*g* for 10 min at 4°C), 500 μl of the supernatants was subjected to a commercially available cAMP radioimmunoassay (Amersham RPA 509, Braunschweig, Germany). The radioimmunoassay uses a ^{125}I-succinyl-cAMP tyrosine methylester tracer, together with a highly specific rabbit anti-succinyl-cAMP serum. The tracer bound to the antibody was separated from free tracer with a second antibody (donkey anti-rabbit serum) coated onto magnetizable polymer particles, which allowed magnetic pelleting. Recoveries were 92.3% (82.5-102.1%; n = 16).

Statistical Analysis

The equilibrium dissociation constants (K_D) and the maximal number of binding sites (B_{max}) were calculated from plots according to the method of Scatchard[25] using GraphPad software (San Diego, CA). Inhibition of ICYP binding by isoproterenol or epinephrine was analyzed by nonlinear least-squares regression under the assumption of a model of one or two independent classes of receptor sites according to the following equations.

For one class of receptor sites we used

$$B = B_{max}/(1 + 10^{(\mathrm{Log}(EC_{50}) - \mathrm{Log}[\mathrm{agonist}]) \times HS}).$$

For two classes of receptor sites we used

$$B = B_{max} \times \mathrm{Frac1}/(1 + 10^{(\mathrm{Log}(EC_{50}(1)) - \mathrm{Log}[\mathrm{agonist1}])}) + B_{max} \times \mathrm{Frac2}/(1 + 10^{(\mathrm{Log}(EC_{50}(2)) - \mathrm{Log}[\mathrm{agonist2}])}).$$

with B = specific radioligand binding, B_{max} = maximal specific binding (set to 100% in our example), EC_{50} = the concentration of agonist that displaces the radioli-

gand from the respective binding site by 50%, [agonist] = the concentration of the displacing agonist, HS = Hill slope, and Frac1 and Frac2 = fractions of the respective binding site compared with the total receptor population.

To review these equations, see the studies by Engel *et al.*[26] and Weiss.[27]

In the radioligand experiments, k_i values were calculated according to the equation of Cheng and Prusoff[28] for each individual experiment. Similarly, EC_{50} values for the increase of force of contraction and the increase in the cAMP level after β-adrenergic stimulation were determined for each experiment by nonlinear least-squares regression analysis.

All data are given as mean with 95% confidence interval.

Statistical significance was determined by two-tailed, unpaired Student's *t* tests. A probability value <0.05 was considered significant.

Materials

Halothane (Hoechst AG, Frankfurt am Main, Germany) or isoflurane (Abbott, Wiesbaden, Germany) vapor was delivered by a Draeger 19 vaporizer or by a Isotec 3 vaporizer (Ohmeda). GTP and isoproterenol were from Boehringer (Mannheim, Germany); epinephrine was from Fluka Biochemicals (Buchs, Switzerland); ^{125}I-Iodocyanopindolol was from New England Nuclear (Dreieich, Germany); cAMP radioimmunoassay RPA 509 was from Amersham-Buchler (Braunschweig, Germany); CGP 12177 was a gift from Ciba Geigy (Basel, Switzerland), medium 199 was from ICN Biomedicals (Costa Mesa, CA); and collagenase was from Wako (Neuss, Germany). All other compounds used were of analytical quality or the best grade commercially available. Deionized or twice-destilled water was used in all experiments.

Results

The Force of Contraction of Rat Papillary Muscles

Halothane and isoflurane exhibited a dose-dependent negative inotropic effect on rat papillary muscles that was maximal after 5 min of exposure and rapidly reversible after the removal of the anesthetic from the carbogen applied to the organ chamber. At 1.3 minimum alveolar concentration (MAC; 1 vol% halothane or 1.7 vol% isoflurane), both anesthetics reduced the force of contraction by approximately 50%. At higher, but equipotent, concentrations, the negative inotropic effect of halothane was more pronounced than that of isoflurane (fig. 1). The experiments on the effects of the anesthetics on the β-adrenergic stimulation of the force of contraction were performed at 1.3 MAC of each anesthetic (*i.e.*, a concentration of anesthetic that reduces contractile force by approximately 50%).

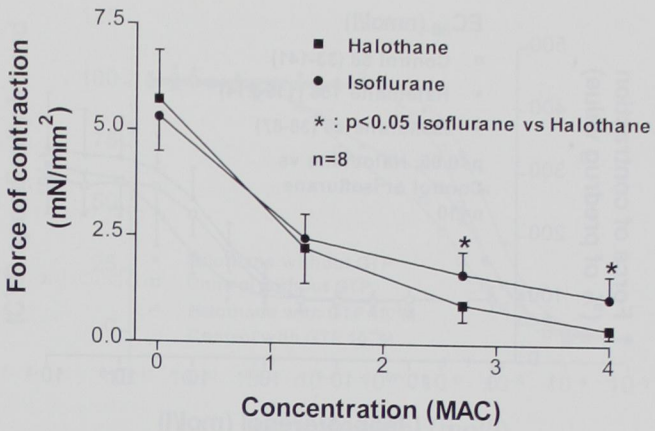

Fig. 1. The negative inotropic effect of halothane (■, 1.3 minimum alveolar concentration [MAC] = 1 vol%, 2.7 MAC = 2 vol%, 4 MAC = 3 vol%) and of isoflurane (●, 1.3 MAC = 1.7 vol%, 2.7 MAC = 3.3 vol%, 4 MAC = 5 vol%) in the absence of inotropes. The forces of contraction without anesthetic were 5.7 mN/mm² (4.5–6.9 mN/mm²) in the halothane group (n = 8) and 5.4 mN/mm² (4.7–6.1 mN/mm²) in the isoflurane group (n = 8, NS). All values are given as means with 95% confidence intervals. Ordinate = The isometric force of contraction of rat papillary muscles in mN/mm²; abscissa = the MAC fraction of anesthetic.

Both catecholamines elicited a threefold increase in the force of contraction. The EC_{50} of isoproterenol was reached at 68 nM (33–141 nM) and that of epinephrine at 0.72 μM (0.44–1.20 μM). Halothane (1.3 MAC) reduced the positive inotropic potency of isoproterenol and epinephrine, whereas the efficacy of the catecholamines was unchanged. In contrast, neither the potency nor the efficacy of isoproterenol or epinephrine were changed in the presence of 1.3 MAC isoflurane. The concentration–response curve of isoproterenol alone, and in the presence of halothane and isoflurane, on the force of contraction is shown in figure 2, and the numeric data are shown in table 1. Figure 3 shows that halothane did not change the negative inotropic effect of carbachol.

The Effect of Volatile Anesthetics on the Agonist Affinity of β-adrenoceptors

Binding of the radioligand ICYP to cardiomyocyte membranes showed saturation characteristics (fig. 4A),

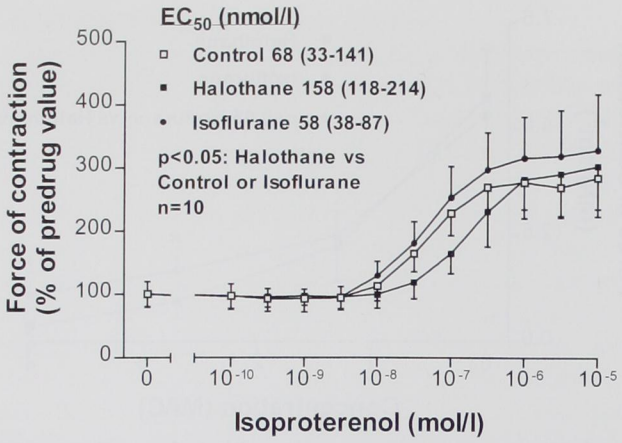

Fig. 2. The concentration–response curves of the effect of isoproterenol alone (□) and with the addition of 1.3 minimum alveolar concentration (MAC) halothane (■) or 1.3 MAC isoflurane (●) on the isometric force of contraction of rat papillary muscles. All values are given as means with 95% confidence intervals. Ordinate = the force of contraction in the percentage of predrug value; abscissa = the molar concentration of isoproterenol. Table 1 shows the basal force of contraction and the increase in the force of contraction.

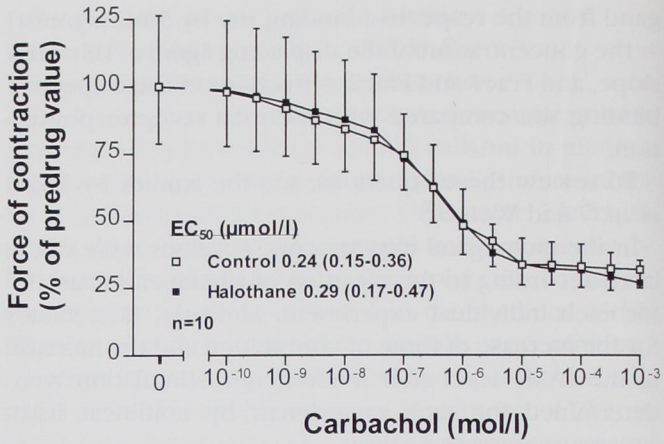

Fig. 3. The concentration–response curve of the effect of carbachol alone (□) and with the addition of 1.3 MAC halothane (●) on the isometric force of contraction of rat papillary muscles. The effect of carbachol was investigated after prestimulation of the muscles with 0.3 µM isoproterenol. Halothane did not modify the potency or efficacy of the negative inotropic effect of carbachol. All values are given with 95% confidence intervals. The forces of contraction before the exposure to carbachol (predrug value) were 15.4 mN/mm² (12.2–18.6 mN/mm²) under control conditions (n = 10) and 8.4 mN/mm² (6.2–10.6 mN/mm²) in the presence of 1.3 MAC halothane (n = 10; $P < 0.05$). Ordinate = the force of contraction in the percentage of predrug value; abscissa = the molar concentration of carbachol.

and the Scatchard plot (fig. 4B) was linear, indicating that ICYP was bound to one distinct receptor binding site. To determine the effect of volatile anesthetics on the agonist affinity of β-adrenoceptors, ICYP was displaced with isoproterenol in the absence of GTP. Figure 5 shows the result of one representative experiment. The competition binding curve was shallow (slope factor = −0.48) and showed two different classes of binding sites, representing the high and the low agonist affinity state of the β-adrenoceptors.[22] In the presence of replaced GTP, the competition binding curve was steeper (slope factor = −1.05) and shifted to the right. Under these conditions it showed only one class of receptor binding sites, and the high-affinity state receptor binding was no longer apparent. Figure 5 also shows the influence of halothane on the competition binding curves of ICYP displacement with isoproterenol. Nonlinear regression analysis allowed us to estimate the dissociation constants (K_i value) and the percentages

Table 1. Concentration–response Curve for Isoproterenol and Epinephrine

Condition	Control	Halothane (1 vol%)	Isoflurane (1.7 vol%)
Isoproterenol			
Basal force of contraction (mN/mm²)	4.4 (3.5–5.3)	1.9 (1.5–2.3)*	2.3 (1.9–2.7)*
Maximal increase in force of contraction (% of basal force of contraction)	285 (232–337)	305 (225–384)	329 (236–421)
Potency (EC_{50} value (nM)	68 (33–141)	158 (118–214)*	58 (38–87)
Epinephrine			
Basal force of contraction (mN/mm²)	4.2 (3.1–5.3)	1.8 (1.4–2.2)*	2.4 (2.0–2.8)*
Maximal increase in force of contraction (% of basal force of contraction)	342 (283–401)	378 (233–523)	356 (268–444)
Potency EC_{50} value (µM)	0.72 (0.44–1.20)	3.80 (2.45–5.75)*	0.87 (0.51–1.44)

* $P < 0.05$ compared with control.

of both receptor affinity states (table 2). The percentage of β-adrenoceptors in the high-affinity state was significantly and dose- dependently decreased from 40.5% (38.9-42%; n = 20) in the control group to 30.4% (26.7-34%; n = 5) in the presence of 3 vol% halothane. The K_i value for ICYP displacement by isoproterenol high-affinity binding was not changed in the presence of halothane. In contrast, the K_i value for isoproterenol binding to the receptors in the low-affinity state increased significantly as a result of halothane exposure, which indicates that halothane reduced the affinity of the low-affinity state of the β-adrenoceptors to the agonist isoproterenol. This effect of halothane was also dose- dependent. In all experiments, in the control and in the anesthetic groups, the addition of GTP completely abolished the occurrence of the high-affinity binding state of β-adrenoceptors, and the reduction of the β-adrenoceptor agonist affinity in the presence of halothane could still be observed. Isoflurane, however, did not modify agonist binding to β-adrenoceptors. Neither the percentage of β-adrenoceptors in the high-affinity state nor the affinity of isoproterenol to β-adrenoceptors were changed in the presence of 1.3 MAC isoflurane. Table 2 summarizes the numeric data of this set of experiments.

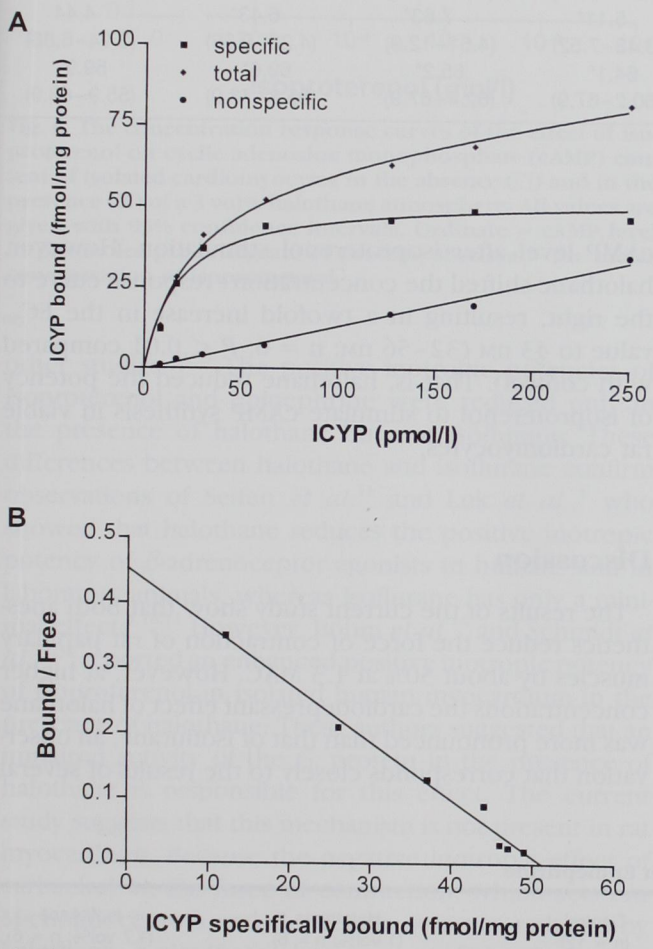

Fig. 4. Representative saturation binding of ^{125}I-Iodocyanopindolol (ICYP) to a rat cardiomyocyte preparation as a function of increasing ICYP concentrations: Binding was performed in the presence of 10^{-4} M guanosine triphosphate at seven concentrations of ICYP ranging from 5–300 pM. Specific binding (■) was determined as the difference of binding in the absence (♦/total binding) and in the presence (●/nonspecific binding) of 1 μM CGP 12177. (*A*) Ordinate = ICYP bound (fmol/mg protein); abscissa = the picomolar ICYP concentration. (*B*) Scatchard analysis of specific ICYP binding of the experiment shown in panel A. The ratio of specifically bound ICYP (in femtomoles per milligram of protein) to free ICYP (picomolar) is plotted as a function of specifically bound ICYP (in femtomoles per milligram of protein).

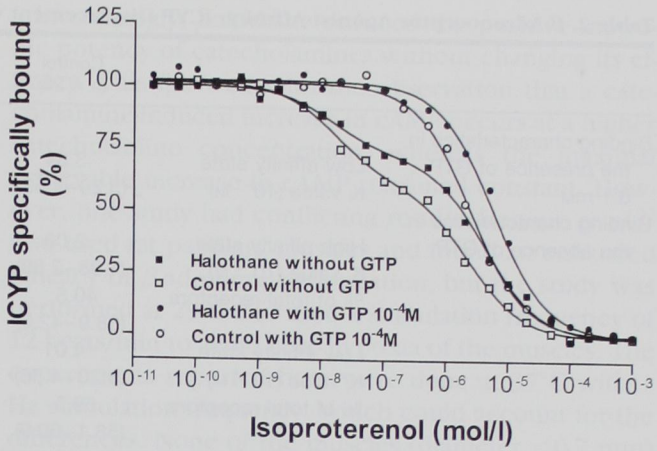

Fig. 5. Representative competition binding curves of ICYP displaced with isoproterenol in the presence (○, ●) and in the absence (□, ■) of guanosine triphosphate (GTP). The open symbols (○, □) show the competition curve in the absence, the closed symbols (●, ■) the competition curve in the presence of 3 vol% halothane. In the absence of GTP β-adrenoceptor, high-affinity binding occurs that cannot be observed in the presence of GTP. Ordinate = ICYP specifically bound in the percentage of total binding; abscissa = the molar concentration of isoproterenol.

The reduction of β-adrenoceptor agonist high-affinity binding and the agonist affinity of the β-adrenoceptor low-affinity state in the presence of halothane could be confirmed by further experiments using epinephrine as a β-adrenergic agonist. As shown in table 3, halothane reduced the percentage of the β-adrenoceptors in the high-affinity state and reduced the affinity of epinephrine to the β-adrenoceptor low-affinity state. The reduction of the agonist affinity of β-adrenoceptor low-affinity state was still present in the presence of GTP.

Table 2. β-Adrenoceptor Agonist Affinity: ICYP Displacement with Isoproterenol

		Control (n = 20)	Halothane (1 vol%, n = 5)	Halothane (2 vol%, n = 5)	Halothane (3 vol%, n = 5)	Isoflurane (1.7 vol%, n = 5)
Binding characteristics in the presence of GTP 0.1 mM	Low affinity state K_i value (10^{-7} M)	3.84 (3.29–4.53)	5.25* (4.68–5.75)	6.17* (3.80–10.23)	7.76* (6.17–9.77)	3.99 (3.11–5.43)
Binding characteristics in the absence of GTP	High affinity state K_i value (10^{-9} M)	2.08 (1.38–2.80)	2.09 (1.38–3.11)	2.63 (2.12–3.44)	1.69 (0.72–3.78)	1.98 (1.29–2.84)
	% of total receptors	40.5 (38.9–42.0)	35.9* (32.0–39.7)	34.8* (32.0–37.5)	30.4* (26.7–34.0)	40.1 (36.1–44.1)
	Low affinity state K_i value (10^{-7} M)	4.01 (3.20–4.36)	5.11* (3.48–7.52)	7.63* (4.51–12.9)	6.43* (4.28–9.66)	4.44 (2.94–6.88)
	% of total receptors	59.5 (58.1–60.9)	64.1* (60.2–67.9)	65.2* (62.4–67.9)	69.6* (65.8–73.0)	59.9 (55.9–63.9)

* $P < 0.05$ compared with control.

Because an impairment of the β-adrenergic responsiveness was observed only with halothane, cAMP levels with isoproterenol stimulation were determined for halothane but not for isoflurane.

Cyclic Adenosine Monophosphate Levels in Viable Rat Cardiomyocytes Exposed to Halothane

Stimulation of adult rat cardiomyocytes with isoproterenol increased the intracellular cAMP level (fig. 6). The basal level of cAMP in the absence of isoproterenol was 40 (35–45) pmol/mg protein. The EC_{50} of isoproterenol was 22 nM (21–23 nM; n = 6), and the effect was maximal at 10 μM. At the maximal isoproterenol concentration, the cAMP level increased about 10 times. The presence of 3 vol% halothane neither altered the basal intracellular cAMP concentration in the rat cardiomyocytes nor the maximal increase in the intracellular cAMP level after isoproterenol stimulation. However, halothane shifted the concentration-response curve to the right, resulting in a twofold increase in the EC_{50} value to 43 nM (32–56 nM; n = 6; $P < 0.01$ compared with control). That is, halothane reduced the potency of isoproterenol to stimulate cAMP synthesis in viable rat cardiomyocytes.

Discussion

The results of the current study show that both anesthetics reduce the force of contraction of rat papillary muscles by about 50% at 1.3 MAC. However, at higher concentrations the cardiodepressant effect of halothane was more pronounced than that of isoflurane, an observation that corresponds closely to the results of several

Table 3. β-Adrenoceptor Agonist Affinity: ICYP Displacement with Epinephrine

		Control (n = 12)	Halothane (1 vol%; n = 6)	Isoflurane (1.7 vol%; n = 6)
Binding characteristics in the presence of GTP 0.1 mM	Low affinity state K_i value (10^{-6} M)	4.70 (4.59–4.92)	8.95* (7.98–10.30)	4.81 (4.30–5.39)
Binding characteristics in the absence of GTP	High affinity state K_i value (10^{-8} M)	3.91 (2.14–4.19)	2.52 (1.03–6.19)	4.49 (3.81–5.27)
	% of total receptors	31.9 (31.0–32.7)	21.6 (20.1–23.1)*	33.2 (30.6–35.8)
	Low affinity state K_i value (10^{-6} M)	4.00 (3.73–4.38)	7.28* (6.34–8.16)	4.38 (4.09–4.70)
	% of total receptors	68.1 (67.2–68.9)	78.4 (76.8–79.9)*	66.8 (64.2–69.3)

* $P < 0.05$ compared with control.

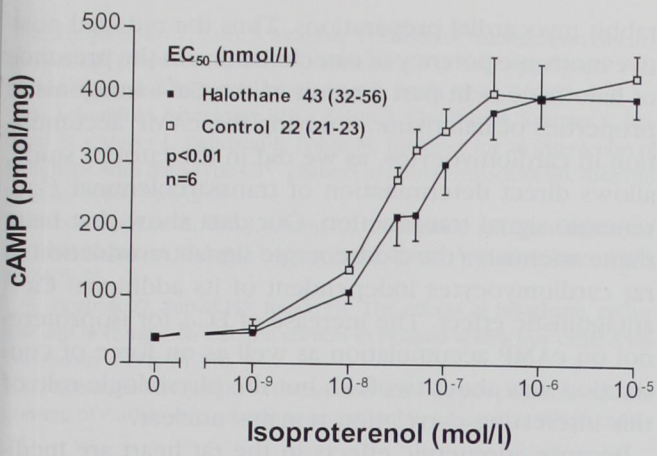

Fig. 6. The concentration response curves of the effect of isoproterenol on cyclic adenosine monophosphate (cAMP) content of isolated cardiomyocytes in the absence (□) and in the presence (■) of a 3 vol% halothane atmosphere. All values are given with 95% confidence intervals. Ordinate = cAMP level in picomoles per milligram of protein; abscissa = the molar concentration of isoproterenol.

other studies.[29,30] The positive inotropic potencies of isoproterenol and epinephrine were reduced only in the presence of halothane but not isoflurane. These differences between halothane and isoflurane confirm observations of Seifen *et al.*[11] and Luk *et al.*,[4] who showed that halothane reduces the positive inotropic potency of β-adrenoceptor agonists in humans and in laboratory animals, whereas isoflurane has only a minimal effect.[4,10,11] However, Böhm *et al.*[16] and Schmidt *et al.*[31,32] reported an enhanced positive inotropic potency of isoproterenol in isolated human myocardium in the presence of halothane. These authors suggested that an impaired activity of the G_i protein in the presence of halothane is responsible for this effect. The current study suggests that this mechanism is not present in rat myocardium. Because the negative inotropic effect of carbachol on the force of contraction, which acts *via* M-cholinoceptors and G_i protein, was not reduced by halothane, a significant impairment of the G_i protein function by halothane in rat cardiomyocytes can be excluded. In addition, an impaired function of G_i protein is expected to result in an increase in adenylyl cyclase activity and subsequently in an increase in the intracellular cAMP level. In contrast, we showed that halothane does not change the basal intracellular cAMP level in rat cardiomyocytes. Perhaps differences in species account for the differences between the results reported by Böhm *et al.*[16] and those obtained in the current and in other investigations.[4,10,11]

Interestingly, halothane reduced the positive inotropic potency of catecholamines without changing its efficacy. This fits well with the observation that a catecholamine-induced increase in cAMP occurs at a higher catecholamine concentration, whereas the maximal achievable increase in cAMP remained constant. However, one study had conflicting results. Hanouz *et al.*[33] also used rat papillary muscles and found an enhanced efficacy of β-adrenergic stimulation, but the study was performed at 29°C and used a stimulation frequency of 12 beats/min to avoid core hypoxia of the muscles. The experiments reported here were done at 37°C with 1 Hz stimulation frequency, which could account for the differences. None of the muscles (diameter <0.7 mm) in the present study showed a decline in the force of contraction >5% when oxygen tension was reduced by 20%, indicating that the contractile performance of the muscles was not limited by oxygen supply.

Only limited data are available that might explain the molecular mechanisms by which halothane, but not isoflurane, reduces the positive inotropic potency of catecholamine and the attenuation of the catecholamine-induced cAMP accumulation in cardiomyocytes. Radioligand binding experiments in the current study showed that halothane, but not isoflurane, shifts the β-adrenoceptor from the high-agonist affinity state to the low-agonist affinity state in a concentration-dependent manner. In addition, halothane reduces the agonist affinity of the β-adrenoceptor in the low-affinity state. These results are independent of the catecholamine (isoproterenol or epinephrine) investigated. Concordant results were reported by Böhm *et al.*,[16] who described halothane inhibiting the high-affinity agonist binding to β-adrenoceptors in human heart membranes. In contrast, Marty *et al.*[15] showed that halothane does not change the isoproterenol affinity of β-adrenoceptors of human lymphocytes. However, the authors of the latter study did not differentiate between the high- and low-agonist affinity binding of the β-adrenoceptors. Further, the reduction of the agonist affinity by halothane that we and Böhm *et al.*[16] observed might be a phenomenon only of β_1-adrenoceptors, representing most of the β-adrenoceptors in the heart, whereas lymphocytes investigated by Marty *et al.*[15] express only β_2-adrenoceptors.

The lack of effect of isoflurane on β-adrenoceptors high- and low-affinity binding is concordant with the finding that isoflurane had no effect on the positive inotropism of isoproterenol or epinephrine.

The high-affinity agonist binding by β-adrenoceptors

has been reported to reflect β-adrenoceptors coupling to the G_s protein. This process is essential for the β-adrenergic signal transmission and was described extensively by De Lean *et al.*[34] in 1983. Briefly, binding of the guanosine diphosphate bound form of the G_s protein to the receptor leads to an increase in the agonist affinity of the receptor. Binding of a catecholamine to the receptor results in the formation of a ternary complex consisting of catecholamine, receptor, and G_s protein. Replacement of guanosine diphosphate by GTP is followed by the dissociation of the ternary complex, and the conformational change of the receptor reduces its agonist affinity, resulting in dissociation of the catecholamine from the receptor. The GTP bound form of G_s protein activates adenylyl cyclase as long as GTP has not been dephosphorylated to guanosine diphosphate by the intrinsic GTPase activity of the G_s protein. During β-adrenergic stimulation of adenylyl cyclase, β-adrenoceptors normally cycle rapidly through the transient high-affinity state in the presence of GTP. After the removal of GTP, the high-affinity state becomes observable in radioligand experiments, as was done in this study. The reduced percentage of β-adrenoceptors in the high-affinity state due to halothane reported here suggests an impairment of G_s protein and receptor coupling or a decrease in G_s protein GTPase activity, both of which indicate an impaired G_s protein function. The impaired G_s protein function, together with the reduced affinity of the low-affinity state of the receptors, may contribute to an attenuation of the β-adrenergic signal transmission by halothane. In close agreement with the radioligand binding data, the potency of isoproterenol to increase the intracellular cAMP level was significantly reduced by halothane. In a previous study[35] we reported that halothane induced no changes in β-adrenoceptor density, subtype distribution, or antagonist affinity with prolonged incubation up to 3 h. Thus changes in β-adrenoceptor density or subtype distribution cannot account for alterations in the β-adrenergic signal transmission during halothane exposure. Therefore we conclude that the direct effect of halothane on β-adrenoceptor agonist binding observed in the current study contributes to an attenuation of β-adrenergic signal transmission.

Volatile anesthetics also have Ca^{2+}-antagonistic properties. They attenuate the voltage-dependent Ca^{2+} inward current[5] and deplete the sarcoplasmic reticulum Ca^{2+} stores.[6,7,36] Bristow and Green[37] showed that compounds with Ca^{2+}-antagonistic properties reduce the positive inotropic potency of isoproterenol in isolated rabbit myocardial preparations. Thus the reduced positive inotropic potency of catecholamine in the presence of halothane is in part a result of the Ca^{2+}-antagonistic properties of halothane. Studying the cAMP accumulation in cardiomyocytes, as we did in the current study, allows direct determination of transsarcolemmal β-adrenergic signal transduction. Our data show that halothane attenuates the β-adrenergic signal transduction in rat cardiomyocytes independent of its additional Ca^{2+} antagonistic effect. The increase of EC_{50} for isoproterenol on cAMP accumulation as well as on force of contraction was about twofold, but the physiologic role of this interesting correlation remains unclear.

Because adrenergic effects in the rat heart are mediated *via* α- and β-adrenoceptors,[33] the reduction of the positive inotropic potency of epinephrine in the presence of halothane could be caused by an interaction of halothane with the α-adrenoceptor–mediated positive inotropism. However, the impairment of the myocardial adrenergic responsiveness could also be observed with isoproterenol, which does not bind to α-adrenoceptors. Therefore we can conclude that halothane interacts specifically with the β-adrenergic signal transduction pathway in addition to the potential alterations of α-adrenergic effects suggested by Hanouz *et al.*[33]

In conclusion, halothane reduces the positive inotropic potency of catecholamines in rat papillary muscles, whereas isoflurane does not alter β-adrenergic stimulation of the force of contraction. Halothane shifts the β-adrenoceptors of isolated adult rat cardiomyocytes from a high-affinity state to a low-affinity state and reduces the agonist affinity of the low-affinity state, indicating that the reduced positive inotropic potency of catecholamines in the presence of halothane is in part a result of reduced β-adrenoceptor agonist affinity. In addition, the transmembrane signal transduction of the β-adrenergic system becomes impaired by halothane, as shown by the reduced potency of the isoproterenol effect on intracellular cAMP accumulation. In contrast, isoflurane does not modify β-adrenoceptor agonist binding or the positive inotropic potency of catecholamines.

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