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Influence of volatile anesthetics on muscarinic regulation of adenylate cyclase activity

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General anesthetics have profound effects on several aspects of cardiovascular performance under autonomic control involving muscarinic cholinergic receptors [1], and it is reasonable to suspect that a disruption of cholinergic transmission in the heart contributes to the development of certain aspects of anesthetic action. We have demonstrated that volatile anesthetics alter the ability of guanine nucleotides to regulate muscarinic agonist binding in both rat cardiac and brainstem membranes, indicating that volatile anesthetics disrupt muscarinic receptor–G protein interactions [2–4]. Halothane was also found to decrease muscarinic control of adenylate cyclase activity [5]. The present studies were designed to determine if disruption of muscarinic modulation of adenylate cyclase activity is a common property of volatile anesthetics. Our results demonstrate that muscarinic control of adenylate cyclase is inhibited by numerous volatile anesthetics.

Materials and Methods

Adult male Wistar rats were killed by decapitation. The hearts were removed and cleaned of fat and blood vessels, and homogenized in TED buffer, pH 7.5 (10 mM Tris–

HCl; 1 mM EDTA; 1 mM dithiothreitol) containing 100 μM physostigmine to inhibit acetylcholinesterase. The homogenates were centrifuged at 20,000 *g* for 10 min at 4°, and the resulting pellets were washed twice with TED buffer and used without further treatment.

Adenylate cyclase activity was measured by the method of Salomon *et al.* [6] with minor modifications. Each assay tube contained the following reagents in a final volume of 250 μL: 25 mM HEPES (*N*-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]), pH 7.5; 1.33 mM EGTA (ethylene glycol-bis(β-aminoethyl ether) *N,N,N',N'*-tetraacetic acid); 1 mM dithiothreitol; 2 mM $MgSO_4$; 50 mM NaCl; 0.1 mM ATP; 1 mM cAMP; 1 μM GTP; 20 mM creatine phosphate; 10 units creatine phosphokinase; 0.5 μCi [^{32}P]ATP (Dupont-NEN); 100–200 μg protein. Assays were performed in quadruplicate. The reaction was started by addition of cardiac membranes, and the incubation was carried out at 37° for 10 min in the continued presence of the anesthetic. The reaction was stopped by immersing the assay tube in a boiling water bath for 1 min followed by centrifuging at 4000 *g* for 15 min. Purification of cAMP was carried out by the method of Mao and Guidotti [7]. An aliquot of the supernatant fraction was loaded onto a neutral alumina column (0.7 × 4 cm) equilibrated with 0.06 M Tris–HCl, pH 7.5, and eluted with 5 mL of the equilibration buffer. The eluate was directly loaded onto an AG 1 × 4 formate (Bio-Rad) column (0.7 × 2 cm) equilibrated with water. The column was washed twice with 10 mL of water, and the bound cAMP was eluted with 5 mL of 1 N formic acid. An aliquot was counted for [^{32}P]cAMP content using a Beckman LS-7000TD liquid scintillation counter. The recovery of cAMP during the purification procedure was routinely monitored using [^{3}H]cAMP. Adenylate cyclase activity was stimulated in some experiments by forskolin or isoproterenol. Membrane suspensions in TED buffer were equilibrated as previously described [5] with one of the following anesthetics: enflurane, isoflurane, ether, or chloroform.

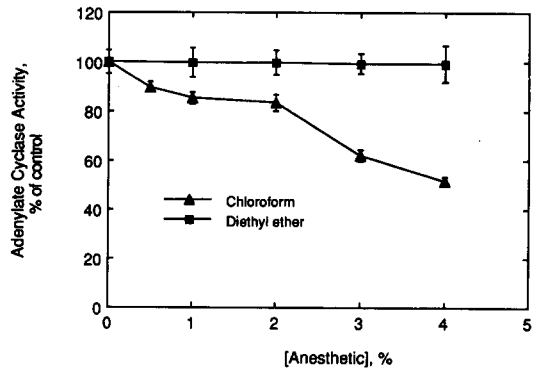

Fig. 1. Influence of volatile anesthetics on basal adenylate cyclase activity in rat cardiac membranes. The formation of cAMP was determined in quadruplicate after equilibrating the membranes with the indicated concentrations of chloroform (▲) or diethyl ether (■). Adenylate cyclase activity is expressed as percent of control basal activity measured in the absence of anesthetic (40–60 pmol/mg protein/10 min at 37° in different preparations). Each value is the mean ± SD from three experiments.

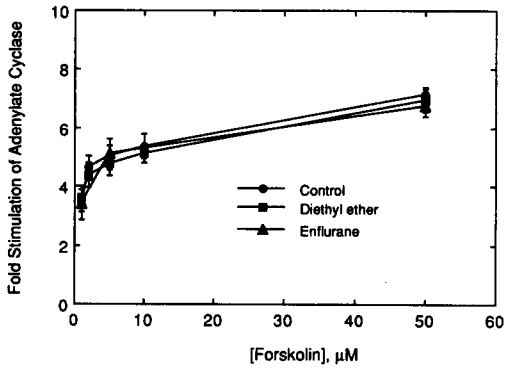

Fig. 2. Influence of volatile anesthetics on the stimulation of adenylate cyclase by forskolin. The formation of cAMP at 37° was measured in the presence of the indicated concentrations of forskolin, and is plotted as fold-stimulation of basal activity. Cyclic AMP formation was measured in membranes equilibrated with air (●), 2% diethyl ether (■), or 2% enflurane (▲). Each value is the mean ± SD from three experiments.

Results

Influence of volatile anesthetics on basal adenylate cyclase activity. Diethyl ether had no effect on basal adenylate cyclase activity in cardiac membranes at concentrations up to 4%, whereas chloroform decreased basal adenylate cyclase activity in cardiac membranes at concentrations greater than 2% (Fig. 1). Like diethyl ether, isoflurane and enflurane had no effect on basal cyclase activity (data not shown). We have demonstrated previously that halothane increases basal adenylate cyclase activity in cardiac membranes [5].

Influence of volatile anesthetics on stimulated adenylate cyclase activity. Forskolin stimulated adenylate cyclase activity in rat cardiac membranes in a concentration-dependent manner (Fig. 2). Adenylate cyclase activity was increased by up to 7-fold in the presence of 50 μM forskolin ($EC_{50} \approx 1$ μM). The degree of enzyme stimulation by forskolin was not affected by diethyl ether or enflurane at 2% (Fig. 2), or by chloroform or isoflurane at 2% (data not shown). Isoproterenol also stimulated adenylate cyclase activity in rat heart membranes by up to 4-fold with an EC_{50} of approximately 0.1 μM; maximum stimulation was obtained in the presence of 10–100 μM isoproterenol (Fig. 3). Essentially similar results were obtained with isoproterenol-stimulated adenylate cyclase as with forskolin-stimulated adenylate cyclase activity; i.e. none of the anesthetics tested affected isoproterenol-stimulated adenylate cyclase activity [e.g. diethyl ether and isoflurane (Fig. 3), and chloroform and enflurane (data not shown)].

Influence of volatile anesthetics on acetylcholine modulation of stimulated adenylate cyclase activity. Acetylcholine, a muscarinic agonist, inhibited adenylate cyclase activity stimulated by 1 μM forskolin in a concentration-dependent manner (Fig. 4). Maximum inhibition (29%) was obtained in the presence of 1 mM acetylcholine. Acetylcholine inhibition was blocked completely by including 10 μM atropine in the assay medium. Diethyl ether and enflurane (2%) attenuated acetylcholine inhibition of forskolin-stimulated adenylate cyclase activity (Fig. 4); isoflurane and chloroform decreasd acetylcholine inhibition of adenylate cyclase activity to a similar extent (data not shown). Acetylcholine also inhibited isoproterenol (100 μM)-stimulated adenylate cyclase activity, maximal inhibition being produced by 100 μM acetylcholine (Fig. 5). This inhibition was diminished by each of the anesthetics at 2% [e.g. diethyl ether and isoflurane (Fig. 5), and chloroform and enflurane (data not shown)].

Discussion

Different volatile anesthetics have different effects on basal adenylate cyclase in cardiac membranes. Chloroform decreased basal adenylate cyclase activity, whereas isoflurane, diethyl ether and enflurane had no effect on basal activity. In contrast, our previous studies demonstrated an increase in basal activity in the presence of halothane [5], in agreement with reports from others [8]. The reasons for

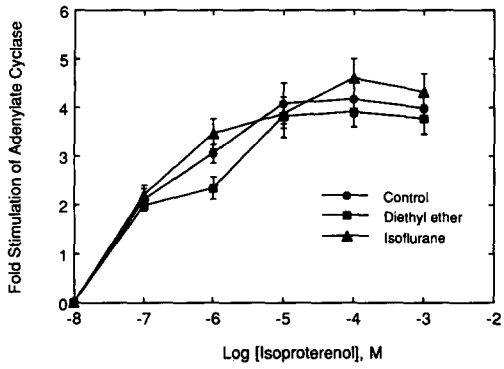

Fig. 3. Influence of volatile anesthetics on the stimulation of adenylate cyclase by isoproterenol. The formation of cAMP at 37° was measured in the presence of the indicated concentrations of isoproterenol, and is plotted as fold stimulation of basal activity. Cyclic AMP formation was measured in membranes equilibrated with air (●), 2% diethyl ether (■), or 2% isoflurane (▲). Each value is the mean ± SD from three experiments.

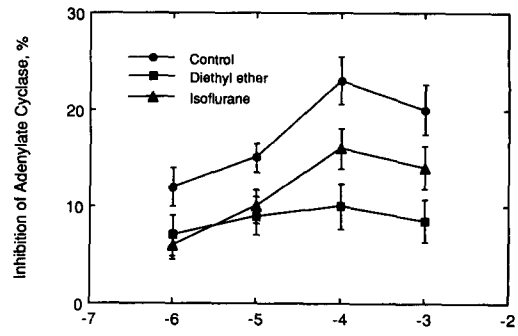

Fig. 5. Influence of volatile anesthetics on acetylcholine inhibition of isoproterenol-stimulated adenylate cyclase activity in rat cardiac membranes. The formation of cAMP was measured in the presence of 100 μM isoproterenol and the concentrations of acetylcholine indicated on the abscissa. Enzyme activity was measured in membranes equilibrated with air (●), 2% diethyl ether (■), or 2% isoflurane (▲). Each value is the mean ± SD from three experiments. Cyclase activity in the absence of anesthetics was 192 ± 14 pmol/mg protein/10 min at 37° (N = 4).

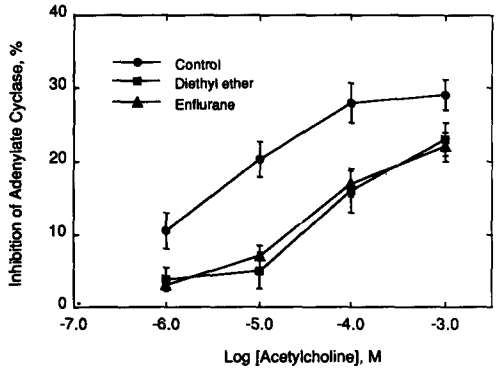

Fig. 4. Influence of volatile anesthetics on acetylcholine inhibition of forskolin-stimulated adenylate cyclase activity in rat cardiac membranes. The formation of cAMP was measured in the presence of 1 μM forskolin and the concentrations of acetylcholine indicated on the abscissa. Enzyme activity was measured in membranes equilibrated with air (●), 2% diethyl ether (■), or 2% enflurane (▲). Each value is the mean ± SD from three experiments. Cyclase activity in the absence of anesthetics was 182 ± 10 pmol/mg protein/10 min at 37° (N = 5).

these differences are not entirely obvious from the chemical structures or physical properties of the anesthetics. The three ether compounds (enflurane, isoflurane and diethyl ether) had no effect on basal activity, whereas one halogenated hydrocarbon (chloroform) decreased and one halogenated hydrocarbon (halothane) increased basal activity. It is interesting that the two anesthetics which affected basal activity (albeit in different directions) possess higher oil/gas partition coefficients than the inactive ethers (224–265 vs 65–96.5 [9, 10]). Thus, the ability to affect basal adenylate cyclase activity is related to the hydrophobicity of the anesthetic. In this regard, it is important to note the recent report by A.G. Gilman (discussed in Ref. 11) that the primary amino acid sequence of adenylate cyclase indicates 12 membrane-spanning regions.

Volatile anesthetics had no effect on forskolin- and isoproterenol-stimulated adenylate cyclase activity in cardiac membranes. These agents stimulate adenylate cyclase by different methods. Forskolin stimulation is a result of an action on the catalytic subunit, perhaps by stabilizing an activated cyclase–G protein complex [12, 13]. Isoproterenol stimulation, on the other hand, is mediated by β-adrenergic receptors via the G_s stimulatory guanine nucleotide dependent-binding protein [14].

Muscarinic cholinergic receptor activation leads to an inhibition of adenylate cyclase activity [15] mediated by a specific guanine nucleotide-dependent regulatory protein, G_i [16]. Each of the volatile anesthetics examined (chloroform, isoflurane, enflurane, and diethyl ether) depressed muscarinic inhibition of stimulated adenylate cyclase activity. These results are consistent with our previous demonstration that halothane decreases carbamycholine inhibition of stimulated adenylate cyclase activity in rat heart [5]. Thus, the effect of volatile anesthetics on receptor control of adenylate cyclase appears to be specific for muscarinic receptors and/or the G_i component of the adenylate cyclase complex, since β-adrenergic receptor stimulation of adenylate cyclase via G_s was not affected. Disruption of muscarinic control of adenylate cyclase activity appears to be a general property of the volatile anesthetics and may play a role in the elaboration of the anesthetic state.

The influence of volatile anesthetics on muscarinic acetylcholine receptor regulation of adenylate cyclase activity was studied using membranes isolated from rat heart. Membranes were equilibrated with enflurane, isoflurane, chloroform, or diethyl ether before measuring adenylate cyclase activity. Diethyl ether, enflurane, and isoflurane had no effect on basal adenylate cyclase activity, whereas chloroform decreased adenylate cyclase activity. The ability of forskolin or isoproterenol to stimulate adenylate cyclase was not altered after equilibration of cardiac membranes with any of the anesthetics. The ability of acetylcholine to modulate stimulated adenylate cyclase activity, however, was depressed after equilibration of the cardiac membranes with each of the anesthetics. These results indicate that the disruption of the muscarinic receptor signalling transduction process is a common property of volatile anesthetics and are consistent with our hypothesis that anesthetics interfere with receptor–G protein interactions.

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The effect of schistosomiasis on the covalent binding of 2-acetylaminofluorene to mouse liver macromolecules *in vivo* and *in vitro*

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Aromatic amines such as 2-acetylaminofluorene (AAF) have been shown to be carcinogenic in many animal species including humans, rodents, rabbits and dogs [1]. AAF undergoes metabolic activation via N-hydroxylation [2, 3] to yield the compound *N*-hydroxy-2-acetylaminofluorene (N-OH-AAF), the proximate carcinogenic metabolite which is sulfated to form the ultimate carcinogen [4]. The cellular nucleophiles that react covalently with the active metabolite of AAF *in vivo* have been identified to be proteins [5, 6], DNA [7] and glycogen [8]. The binding of AAF to microsomal protein and nuclear DNA *in vitro* has also been demonstrated [9, 10]. It is the formation of the carcinogen-macromolecular adducts that is thought to lead to the toxic and carcinogenic effects of the compound.

The response of animals to carcinogens may be modified by parasitic infection. Infected animals develop increased susceptibility to toxins and neoplasia [11–13]. To investigate the effect of parasite infection on metabolic activation of chemical carcinogens, we monitored the binding of AAF to macromolecules in schistosome-infected and non-infected mice both *in vivo* and *in vitro*.

Materials and Methods

Treatment of animals. Eight-week-old BALB/c mice were randomly divided into two groups. One group was infected with 30 *Schistosoma mansoni* cercariae per mouse using the method of Moore *et al.* [14] while the other group served as the control. After infection, the two groups were