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Action of Halothane on Myocardial Adenylate Cyclase of Rat and Cat[1] (40409)

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The cardiodepressant effect of halothane (1) and its antagonism of the inotropic effect of catecholamines is well documented (2–4); however, the mechanisms involved in the action of anesthetics on myocardial contractility are not fully understood. It has been suggested that the positive inotropic effect of catecholamines is mediated by elevated cAMP levels produced by activation of adenylate cyclase (5). Since halothane has been shown to affect the activity of adenylate cyclase in smooth muscle (bronchus (6), uterus (7, 8), blood vessel (9)), liver (10), brain (11), and platelets (12) the purpose of the present study was to determine the effect of halothane on the activity of myocardial adenylate cyclase and its interaction with the effect of catecholamines and of compounds known to alter adenylate cyclase activity by actions exerted at other sites of the enzyme complex.

Methods and materials. Left ventricular muscle was obtained from Sherman rats (150–200 g) and cats (2.0–2.5 kg). The rats were decapitated and the hearts were quickly excised and placed in cold saline. The cat hearts were excised under anesthesia with pentobarbital, 30 mg/kg, ip. The left ventricle was dissected free of all visible fibrous and adipose tissue and thoroughly rinsed with cold saline to remove blood. Immediately before the assay of enzyme activity, approximately 150 mg of the muscle tissue was homogenized with 30 vol of 0.05 *M* Tris HCl buffer (pH 7.5) at 4° in an all-glass, motor-driven homogenizer. Adenylate cyclase activity was determined in the whole tissue homogenate by the method of Krishna (13) measuring the rate of formation of cAMP derived from labeled ATP, modified by the addition of an ATP regenerating system (phosphoenolpyruvate coupled with pyruvate kinase) to maintain a constant ATP concentration and of an excess of unlabeled cAMP to minimize the breakdown of labeled cAMP by phosphodiesterase. In addition, the assay mixture (final vol of 300 μl) contained 1 m*M* ATP in the presence of 3 m*M* $MgSO_4$ and 3.5–5.0 μCi ^{32}P-ATP and, when appropriate, the drug under study. The reaction was started by the addition of 150 μl of tissue homogenate (with a protein content of approximately 75–100 μg). The mixture was incubated at 37° for 5 min and the reaction was stopped by immersion of the test tubes in boiling water for 5 min. Cyclic AMP was isolated by column chromatography using an ion-exchange resin (Dowex 50 W-X4, H^+ form). Trace amounts of contaminating nucleotides were removed by precipitation with zinc sulfate and barium hydroxide. Radioactivity of the cAMP-containing fraction was measured in an Intertechnique liquid scintillation spectrometer, using Bray's mixture. The recovery of cAMP from the column, determined spectrophotometrically at 250 nm in an aliquot of this fraction, was 80–90%. Protein was determined by the method of Lowry *et al.* (14). The activity of adenylate cyclase, corrected for recovery, is expressed in pmol cAMP formed per mg protein per unit of time, calculated from the specific activity of substrate ATP. With each set of measurements, a reagent blank was carried through the procedure with heat-denatured enzyme to correct for any radioactivity not related to enzyme activity. All determinations were made in duplicate.

Halothane vapor was carried in 100% oxygen to the reaction tubes for 20 min before and during the incubation. The concentration of halothane delivered to the liquid mixture

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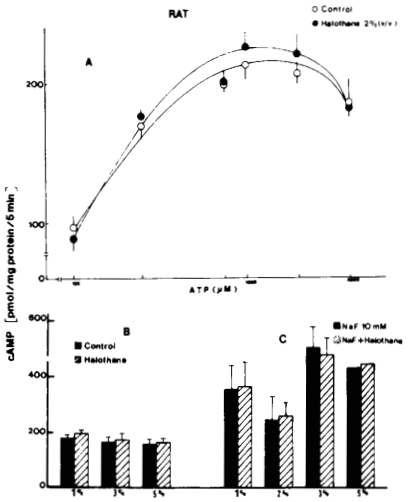

FIG. 1. Adenylate cyclase activity in homogenates of rat left ventricle. A. At increasing concentrations of ATP (maintaining ATP:Mg^{++} ratio constant at 1:3) in the absence (open circles) or presence (closed circles) of halothane 2%, vol/vol. Values are means ± SEM of four experiments. B. At 1 mM concentration of ATP in the absence (black columns) or presence (hatched columns) of halothane 1–5%, vol/vol. Values are means ± SEM of four experiments. C. At 1 m*M* concentration of ATP in the presence of 10 m*M* sodium fluoride alone (black columns) or with halothane 1–5%, vol/vol (hatched columns). Values are means ± SEM of one to three experiments.

was determined by gas chromatography and a Narkotest-M Analyzer. 100% oxygen was used for concurrent controls.

The data were analyzed by a two-way fixed effects analysis of variance for randomized blocks (15). The fixed effects were (a) the presence or absence of halothane and (b) the concentration of the other drug under consideration. This analysis tests (F statistics) the effect of halothane on adenylate cyclase activity, on the response of the enzyme to catecholamines and 5-guanylyl imidodiphosphate in the presence and absence of halothane.

Alpha ^{32}P-ATP and ^{3}H-cAMP were obtained from the New England Nuclear Corp.; cAMP from CalBioChem; ATP, pyruvate kinase and phosphoenolpyruvate, from the Boehringer-Mannheim Corp.; 5-guanylyl imidodiphosphate (Gpp(NH)p), from ICN Pharmaceuticals, Inc.; *dl*-isoproterenol HCl and *l*-norepinephrine bitartrate, from Winthrop Laboratories.

Results. Effect of halothane on basal (non-stimulated), sodium fluoride- and Gpp(NH)p-induced adenylate cyclase activity. The basal activity of rat myocardial adenylate cyclase and the sodium fluoride-induced activity were not affected by halothane (0.5–5%, vol/vol). The myocardial adenylate cyclase activity was not different in the absence and presence of halothane (2%, vol/vol) at all points along the substrate concentration response curve, including that at which the activity was maximal (Fig. 1).

Gpp(NH)p activated adenylate cyclase in rat myocardial homogenates in a dose-related manner, reaching an increase of 68% above the basal value at 0.2 m*M* concentration. The effect of Gpp(NH)p was significantly reduced by halothane; the effect of the highest dose of Gpp(NH)p was lower, by 23% ($P < 0.001$), in the presence of halothane (2%, vol/vol) (Fig. 2).

Effect of halothane on catecholamine-induced adenylate cyclase activity. Norepinephrine, as shown previously by others (5), had a dose-dependent stimulatory effect on rat myocardial adenylate cyclase activity, with a maximum increase of 82.0 ± 5.0 pmol/mg

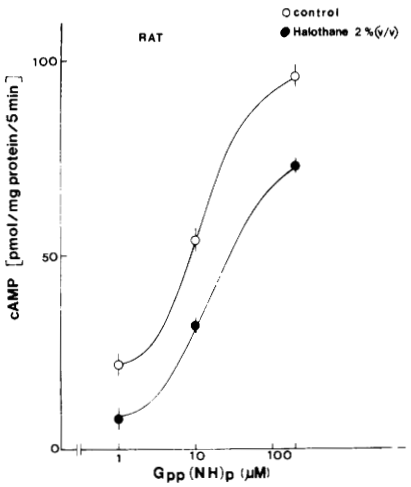

FIG. 2. Effect of Gpp(NH)p on adenylate cyclase activity in homogenates of rat left ventricle. Open circles: mean net increase ± SEM above basal activity (140.0 ± 7.0 pmol cAMP/mg protein/5 min). Closed circles: mean net increase ± SEM in the presence of halothane 2%, vol/vol (halothane alone: 140.0 ± 3.0 pmol cAMP/mg protein/5 min). Net effects of Gpp(NH)p in the absence and presence of halothane are significantly different at all concentrations ($P < 0.001$). $n = 5$.

protein/5 min above the basal value (140.0 ± 1.0 pmol/mg protein/5 min) reached at 10 μM concentration. In the presence of halothane, the adenylate cyclase-stimulatory effect of norepinephrine was significantly diminished at all concentrations of norepinephrine, including the maximum effect, which was decreased by 24 and 56% in the presence of halothane 2 and 3% (vol/vol), respectively ($P < 0.001$). The ED_{50} of norepinephrine, estimated from the dose-response curve, did not change markedly in the presence of halothane (Fig. 3).

Isoproterenol, 0.75–5.0 μM, increased the activity of adenylate cylase in the rat myocardial homogenate in a dose-dependent manner, with the maximum effect obtained at 5 μM concentration; an increase of 136.0 ± 11.0 pmol/mg protein/5 min above the basal value (184.0 ± 12.0 pmol/mg protein/5 min). Halothane decreased the effect of isoproterenol on adenylate cyclase significantly all along the dose-response curve. The maximum effect of isoproterenol was 99.0 ± 13.0

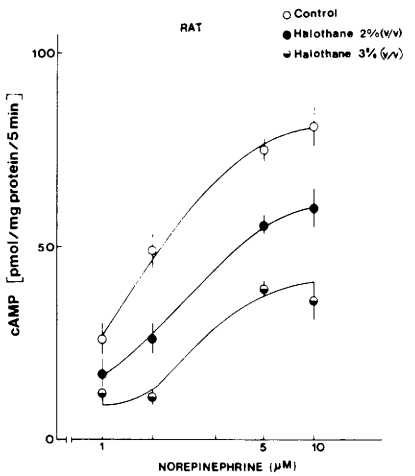

FIG. 3. Effect of norepinephrine on adenylate cyclase activity in homogenates of rat left ventricle. Open circles: mean net increase ± SEM above basal activity (140.0 ± 1.0 pmol cAMP/mg protein/5 min). Closed circles: mean net increase ± SEM in the presence of halothane 2%, vol/vol (halothane alone: 142.0 ± 2.0 pmol cAMP/mg protein/5 min). Semi-closed circles: mean net increase ± SEM in the presence of halothane 3%, vol/vol (halothane alone: 133.0 ± 6.0 pmol cAMP/mg protein/5 min). Net effects of norepinephrine in the absence and presence of halothane are significantly different at all concentrations ($P < 0.001$). $n = 4$.

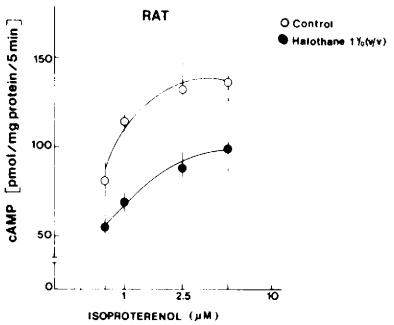

FIG. 4. Effect of isoproterenol on adenylate cyclase activity in homogenates of rat left ventricle. Open circles: mean net increase ± SEM above basal activity (184.0 ± 12.0 pmol cAMP/mg protein/5 min). Closed circles: mean net increase ± SEM in the presence of halothane 1%, vol/vol (halothane alone: 196.0 ± 10.0 pmol cAMP/mg protein/5 min). Net effects of isoproterenol in the absence and presence of halothane are significantly different at all concentrations ($P < 0.001$). $n = 5$.

pmol/mg protein/5 min above the basal value (196.0 ± 10.0 pmol/mg protein/5 min) in the presence of halothane 1% (vol/vol), 27% less than the effect of isoproterenol alone ($P < 0.001$) (Fig. 4). Halothane 2% (vol/vol) decreased the maximum effect of isoproterenol on rat myocardial adenylate cyclase by 63% ($P < 0.001$); from 71.0 ± 8.0 above the basal value of 175.0 ± 10.0 pmol/mg protein/5 min in the absence of halothane to 26.0 ± 6.0 above the activity in the presence of halothane 2% (vol/vol), 187.0 ± 6.0 pmol/mg protein/5 min ($N = 4$, isoproterenol 2.5 μM).

In the cat myocardium, the maximum net effect of isoproterenol (255.0 ± 6.0 above a basal activity of 290.0 ± 5.0 pmol/mg protein/5 min, an increase of 87%) was reached at a concentration of 5 μM. This effect of isoproterenol was decreased by 22% ($P < 0.001$) in the presence of halothane (2%, vol/vol) (Fig. 5).

Discussion. The present results show that halothane, an inhalation anesthetic with cardiodepressant effect, inhibits the catecholamine-stimulated adenylate cyclase in the heart without affecting the basal, non-stimulated, or sodium fluoride-induced activity of the enzyme. The action of halothane on adenylate cyclase in this tissue is different from that observed in other organs with the exception, perhaps, of toad bladder. Halothane has

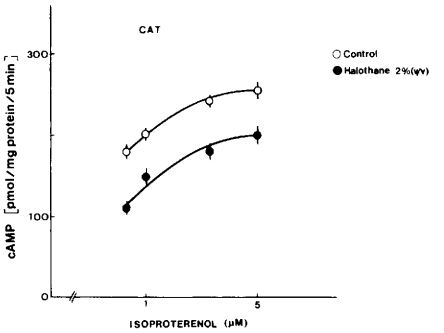

FIG. 5. Effect of isoproterenol on adenylate cyclase activity in homogenates of cat left ventricle. Open circles: mean net increase ± SEM above basal activity (290.0 ± 5.0 pmol/mg protein/5 min). Closed circles: mean net increase ± SEM in the presence of halothane 2%, vol/vol (halothane alone: 313.0 ± 6.0 pmol cAMP/mg protein/5 min). Net effects of isoproterenol in the absence and presence of halothane are significantly different at all concentrations ($P < 0.001$). n = 3.

been shown to increase basal adenylate cyclase activity in smooth muscle (6–9), liver (10), brain (11), and platelets (12), and to enhance the effect of isoproterenol and prostaglandin E_1 on uterine adenylate cyclase (7). On the other hand, the results of the study of Levine *et al.* (16, 17) suggest that halothane may have an effect on adenylate cyclase in the toad bladder similar to that observed in the myocardium. These investigators found that methohexital, methoxyflurane, and halothane selectively inhibited vasopressin-stimulated water flow in the toad bladder and that methohexital and methoxyflurane inhibited vasopressin-stimulated adenylate cyclase activity without affecting the basal and sodium fluoride-induced activity of the enzyme.

It has been proposed that some of the effects of halothane on the cAMP-generating enzyme are related to the pharmacological properties of this agent. It has been demonstrated that the activating effect of halothane on adenylate cyclase in bronchial (6) and uterine (7, 8) smooth muscle results in a higher rate of cAMP formation and is accompanied by a concentration-dependent relaxing effect of the isolated rat tracheal and uterine strip. Sprague *et al.* (9) have shown that halothane increases the rate of cAMP formation and inhibits the phenylephrine-induced contraction of the rat aortic strip and have postulated that the decrease in the contractile response due to halothane may be related to its effect on the cAMP system. Presently available evidence suggests that the positive inotropic effect of catecholamines is mediated by cAMP (5). The importance of an antagonism between halothane and norepinephrine in the mechanism of the hemodynamic changes during halothane anesthesia is indicated by the following observations. In the toad heart a decreased responsiveness to norepinephrine was observed in the presence of halothane (2). In man, halothane anesthesia was shown to be associated with diminished pressor response to norepinephrine (3). Similarly, Miller and Morris (4) found stronger circulatory depression in dogs during halothane anesthesia. It is interesting to note that the myocardial depressive effects of halothane and of a barbiturate, thiamylal, in isolated dog myocardium could be reversed by exogenous dibutyryl cAMP (18). It is therefore possible to speculate that the inhibition of the catecholamine-stimulated adenylate cyclase by halothane and the diminished inotropic effect of catecholamines observed in the presence of halothane are related. However, the determination of the cAMP content in intact preparations *in vitro* together with the functional effects of catecholamines as well as *in vivo* in the absence and presence of halothane is necessary in order to test further such a hypothesis. On the other hand, the finding that halothane had no effect on the basal activity of myocardial adenylate cyclase would suggest that the cardiodepressant effect of halothane alone is probably not exerted through the cAMP system.

The results showing the interaction of halothane with compounds known to, or assumed to, act at different sites on the adenylate cyclase enzyme complex suggest possible sites of halothane action. Adenylate cyclase is thought to consist of three subunits: the regulatory unit, facing the outside of the cell, which carries specific receptors; the catalytic unit, oriented towards the inside of the cell, which converts ATP to cAMP; and the third component, a coupling unit, which links, at least functionally, the regulatory and the catalytic units (19). The fact that halothane has no effect on myocardial adenylate cyclase

activity at increasing ATP concentrations indicates that the catalytic unit is not the site of halothane action. This is also supported by the observation that halothane does not alter the response of adenylate cyclase to sodium fluoride, a compound thought to act on the catalytic unit (20). The stimulatory effect of norepinephrine and isoproterenol on myocardial adenylate cyclase, exerted at specific receptor sites, was significantly decreased by halothane. The maximum response to each agonist tested was diminished while the affinity of catecholamines for the enzyme did not seem to be altered in the presence of halothane as there was no marked change in ED_{50}. That halothane does not act through β-adrenergic receptors is also suggested by observations of others. Flacke and Alper reported that norepinephrine can overcome the cardiodepressant effect of halothane only at lower concentrations of the anesthetic and suggested a physiologic type of antagonism and proposed that the two drugs do not interact at the same receptors (21). Yang *et al.* (8) and Triner *et al.* (7) concluded from their recent studies that the activation of adenylate cyclase by halothane in smooth muscle is not due to a β-adrenergic-like action since β-adrenergic blocking compounds did not alter the halothane-induced activation of adenylate cyclase nor its relaxing effects. Although it appears that halothane does not act directly on the β-adrenergic receptors, alteration in the transfer of the hormonal signal by halothane in the myocardium, due to a change in the properties of the regulatory unit such as decrease in available binding sites cannot be excluded. Another site of halothane action—the link between the regulatory and the catalytic unit—is suggested by the halothane-induced decrease of the Gpp(NH)p effect, since this compound increases the activity of adenylate cyclase and its response to hormones by an action probably exerted at a site coupling the regulatory and catalytic unit (19). Consequently, the diminished adenylate cyclase response to agonists in the presence of halothane could result from an impairment of the transmission of the signal from the regulatory to the catalytic unit.

Further work is needed to clarify the exact mechanism of action of halothane on myocardial adenylate cyclase, to see whether other anesthetics depressing myocardial contractility have similar effects and to establish whether there is indeed a cause-effect relationship between the action of halothane on adenylate cyclase and changes in myocardial contractility.

Summary. The effect of halothane on myocardial adenylate cyclase was studied since cAMP is thought to participate in the regulation of myocardial contractility and since halothane has been shown to alter the activity of the cAMP-generating enzyme in other organs. Halothane significantly decreased the stimulatory effect of catecholamines on myocardial adenylate cyclase without altering the basal or the sodium fluoride-induced adenylate cyclase activity. In the presence of halothane (2%, vol/vol) the effect of 5 μM norepinephrine on adenylate cyclase in rat myocardium, 74.0 ± 3.0, was reduced to 53.0 ± 3.0 pmol/mg protein/5 min ($P < 0.001$) (control adenylate cyclase activity, 140.0 ± 1.0 pmol/mg protein/5 min). The activation of adenylate cyclase by isoproterenol was also significantly decreased by halothane in rat and cat myocardium. The maximum stimulatory effect of isoproterenol (2.5 μM) on adenylate cyclase in rat myocardium, 71.0 ± 8.0, was decreased to 26.0 ± 6.0 pmol/mg protein/5 min ($P < 0.001$) in the presence of halothane (2%, vol/vol). Furthermore, halothane diminished the effects of 5-guanylyl imidodiphosphate, which is thought to act at a site linking the regulatory and catalytic units of adenylate cyclase. The results suggest possible sites of halothane action on myocardial adenylate cyclase and raise the possibility that this inhibitory action of halothane and the decreased positive inotropic action of catecholamines in the presence of halothane may be related.

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