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SHORT COMMUNICATION

The Action of Halothane on Adenylate Cyclase

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SUMMARY

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Halothane, at concentrations of from 1-10 volume %, significantly increased adenylate cyclase activity in rat uterine homogenates. The activation of adenylate cyclase by halothane was not altered by propranolol. The inhibitory effect of this *beta* adrenergic blocking compound on isoproterenol-stimulated adenylate cyclase was not changed by halothane, while the stimulation of adenylate cyclase by isoproterenol, including the maximally effective concentration, was enhanced. The activation of the enzyme by other agonists—prostaglandin E_1, sodium fluoride, and 5'-guanylylimidodiphosphate—was also markedly increased by halothane at each concentration of the agonists tested, including maximally effective concentrations. It is proposed that halothane exerts its action on uterine adenylate cyclase through conformational changes of the catalytic unit, resulting in a higher activity of the enzyme.

The mechanisms through which halothane, one of the most commonly used anesthetics, exerts its pharmacological effects are not well understood. Recently we have reported that halothane increases adenylate cyclase activity in rat uterus and proposed that some of its pharmacological effects, namely, its smooth muscle-relaxing properties, may be related to its action on adenylate cyclase (1). Subsequently, similar effects of halothane, documented either by an increase in adenylate cyclase activity or by an increase in the rate of cAMP[1] formation, were demonstrated in preparations of rat aorta (2), bronchus (3), neuroblastoma cells (4), brain (5), and liver (6); however, the mechanism of action of halothane on adenylate cyclase is still unknown. The present study was undertaken in an attempt to clarify this action of halothane.

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[1] The abbreviation used is: cAMP, adenosine cyclic 3',5'-monophosphate.

Adenylate cyclase activity was measured in whole rat uterine homogenates by a modification of the method of Krishna (7, 8). Halothane vapor was delivered by an Ohio-Heidbrick anesthetic machine, carried by 100% oxygen (total flow, 4 liters/min), water-saturated, and distributed through polyethylene tubing and a metal manifold to the test tubes. The assay mixture was previously exposed at 0-4° (to prevent ATP degradation) for 20 min to the anesthetic at concentrations used during the incubation, which was started by the addition of the enzyme source and allowed to proceed for 5 min at 37°. Control samples were handled in the same manner, equilibrated with 100% oxygen at identical flow rates. The concentration of halothane delivered was measured by a

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Drager Narkotest-M anesthesia analyzer and a Perkin-Elmer vapor fractometer calibrated with Ohio Medical Products standards. The results are expressed as picomoles of cAMP per milligram of protein per 5 min, calculated from the specific activity of the substrate labeled with [^{32}P]ATP. The significance of the results was evaluated by Student's *t*-test for paired samples.

The concentration-dependent increase in rat uterine adenylate cyclase activity induced by halothane was significant ($p < 0.0025$) at all concentrations tested and reached the maximum at about 10 volume % (Fig. 1). Complete reversal of the activation of the enzyme occurred within 20 min following the exposure to halothane. When adenylate cyclase activity was determined as a function of substrate concentration with the Mg^{++}:ATP ratio maintained at 3:1, the maximum activity of the enzyme (at an ATP concentration of 2 mM) in the presence of 3% halothane was increased from 200.5 to 253.0 pmoles of cAMP per milligram of protein per 5 min, without apparent change in the affinity for the substrate.

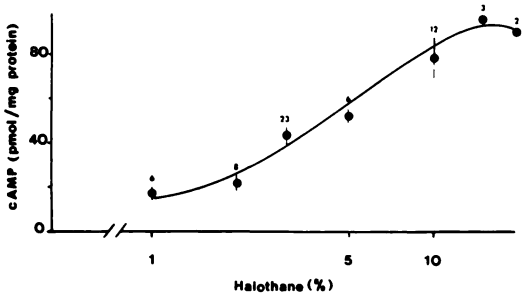

FIG. 1. *Adenylate cyclase activity in rat uterine homogenates in response to halothane*

The assay mixture (total volume, 300 μl) consisted of Tris-HCl buffer, 50 mM, pH 7.5; ATP, 1 mM, with approximately 3.5 μCi of [^{32}P]ATP (2-20 Ci/mmole); $MgSO_4$, 3 mM; phosphoenolpyruvate, 3.3 mM; pyruvate kinase, 15 units/ml; cAMP (unlabeled), 5 mM; and the enzyme source containing approximately 500 μg of protein. The incubation was carried out at 37° for 5 min. Points represent mean net increases ± standard errors above control (72.4 ± 7.6 pmoles of cAMP per milligram of protein per 5 min). Numerals above the points represent the number of experiments.

The *beta* adrenergic blocking compound propranolol did not alter the effect of halothane on adenylate cyclase (increases of 48.6 ± 2.4 and 47.5 ± 5.2 pmoles of cAMP per milligram of protein per 5 min with 3% halothane alone and in the presence of 0.1 μM propranolol, respectively), while the maximum effect of isoproterenol (5 μM), 30.3 ± 2.5, was decreased in the presence of propranolol to 14.2 ± 2.2 pmoles/mg of protein per 5 min ($p < 0.001$). Halothane did not alter the inhibitory effect of propranolol on isoproterenol-induced adenylate cyclase activity.

The response of adenylate cyclase to isoproterenol was increased in the presence of halothane at each concentration of isoproterenol tested ($p < 0.025$) (Fig. 2A). The stimulatory effect of prostaglandin E_1 on uterine adenylate cyclase was increased in the presence of halothane in a similar manner as was the effect of isoproterenol (Fig. 2B). This effect of halothane is characterized by a higher maximum response of adenylate cyclase to isoproterenol and prostaglandin E_1 without an apparent change in affinity for the enzyme. The maximum effect of sodium fluoride (10 mM)—a compound which, in contrast to isoproterenol and prostaglandin E_1, most likely acts on the catalytic unit of the enzyme (9)—was increased by 2% halothane from 101.4 ± 4.6 to 144.5 ± 5.3 pmoles of cAMP per milligram of protein per 5 min ($p < 0.001$), indicating that the fluorine moiety of the halothane molecule is not a carrier of the effect on adenylate cyclase. This is further supported by the effect of pyrophosphate (3 mM), which completely abolished the sodium fluoride (10 mM) activation of adenylate cyclase, while it decreased the responses to 5% halothane and 10 mM isoproterenol by only 50% and 59%, respectively. The stimulation of adenylate cyclase induced by 5'-guanylylimidodiphosphate at 1 and 10 μM (the latter representing the maximally effective concentration) was also significantly ($p < 0.05$) increased by halothane (Fig. 3).

Earlier pharmacological studies suggested that halothane may have a *beta*

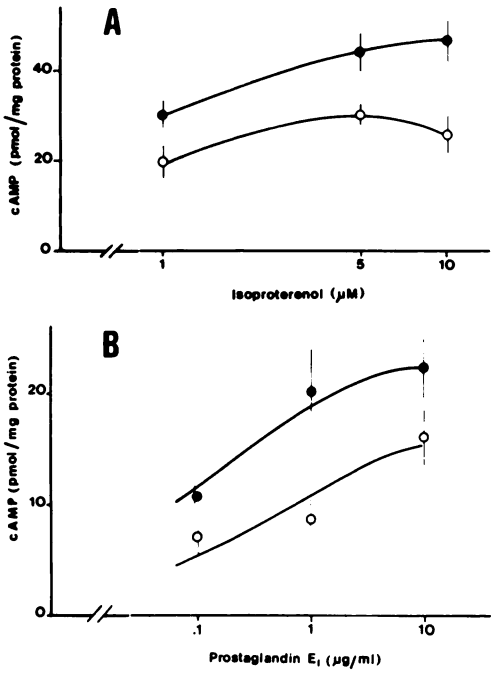

FIG. 2. *Adenylate cyclase activity in rat uterine homogenates in response to isoproterenol and prostaglandin E_1 in the absence and presence of halothane*

A. O——O, net effect of isoproterenol above basal activity (65.0 ± 5.3 pmoles of cAMP per milligram of protein per 5 min); ●——●, net effect of isoproterenol in the presence of 3% halothane (halothane alone, 81.9 ± 14.5 pmoles/mg of protein per 5 min). Values are means and standard errors of eight experiments.

B. O——O, net effect of prostaglandin E_1 above basal activity (67.2 ± 7.0 pmoles/mg of protein per 5 min); ●——●, net effect of prostaglandin E_1 in the presence of 3% halothane (halothane alone, 103.2 ± 8.3 pmoles/mg of protein per 5 min). The difference between the net effects of prostaglandin E_1 is significant at 1 μM concentration ($p < 0.005$). Values are means and standard errors of eight experiments.

adrenergic-like action in smooth muscle (10–12). Although not confirmed by results of recent studies using *beta* adrenergic blocking compounds (13–15), such a possibility of halothane action on adenylate cyclase was explored. The increased adenylate cyclase response to isoproterenol in the presence of halothane, including that at maximally effective concentrations of isoproterenol, and the observation that there was no alteration of halothane effect on adenylate cyclase by propranolol and no change in the inhibitory effect of propranolol on isoproterenol stimulation of adenylate cyclase by halothane suggest that *beta* adrenergic receptors are not involved in the activation of adenylate cyclase by halothane. This is further supported by the synergistic effect of halothane with prostaglandin E_1, which presumably acts through different receptor sites. The higher response of adenylate cyclase to isoproterenol and prostaglandin E_1 in the presence of halothane, characterized by a higher maximum effect without apparent change in the affinity of the compounds for the enzyme, may indicate a higher number of receptors or a faster rate of receptor-drug interaction. However, an action of halothane at or on the catalytic unit of the enzyme seems more likely, since the response of adenylate cyclase to 5′-guanylylimidodiphosphate and sodium fluoride, compounds that presumably act beyond the regulatory unit (9), also increased in the presence of halothane. Such a possibility is supported by the higher V_{max} of the enzyme observed in the presence of halothane.

Halothane and other anesthetics have been shown to produce conformational changes in lipoprotein and phospholipid structures of the cell membrane (16–18). One could speculate, then, that halothane, in this preparation, exerts its action through conformational changes, leading, for example, to alteration in ionic repulsion or steric conformation, resulting in a higher activity of the enzyme. In such a case both the enzyme response to receptor activation and basal, nonstimulated, activity would be higher. Further studies are necessary to test the proposed mechanism of halothane action on adenylate cyclase, including studies with other preparations. The effect of other volatile anesthetics on adenylate cyclase and on other membrane-bound enzymes should also be explored in order to determine whether or not this action is unique to halothane and adenylate cyclase.

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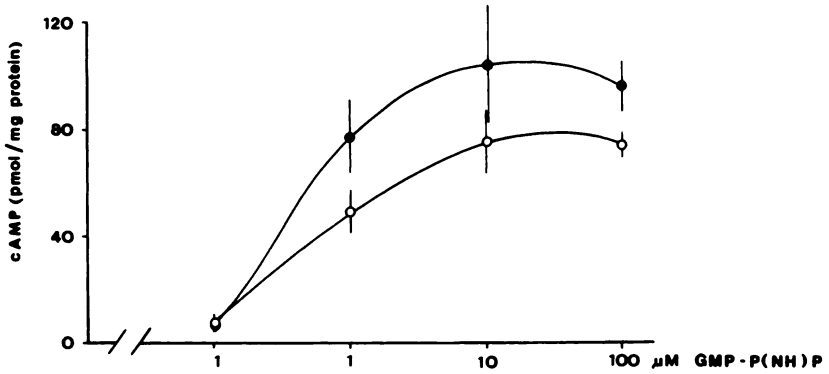

FIG. 3. *Adenylate cyclase activity in rat uterine homogenates in response to 5'-guanylylimidodiphosphate [GMP-P(NH)P]*

○——○, net effect of 5'-guanylylimidodiphosphate above basal activity (79.7 ± 14.5 pmoles/mg of protein per 5 min); ●——●, net effect of the nucleotide in the presence of 3% halothane (halothane alone, 118.2 ± 21.4 pmoles/mg of protein per 5 min). Values are means and standard errors of four experiments.

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