LABORATORY REPORT

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The Effects of Halothane on the Human Beta-adrenergic Receptor of Lymphocyte Membranes

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The effects of halothane on beta-adrenergic receptor antagonist interaction were studied using the membranes of human lymphocytes as a model. Membrane preparations of lymphocytes were obtained from blood samples withdrawn from seven healthy young volunteers. Betareceptor studies were performed using (−)^{125}I iodocyanopindolol (^{125}ICP) binding. Non-specific binding was determined in the presence of (−) isoproterenol. Betareceptor density (Bmax) and the dissociation constant (K_D) for ^{125}ICP were determined from saturation curves. Betareceptor affinity for agonists evaluated by the IC_{50} (the concentration of isoproterenol required to inhibit 50% of specific ^{125}ICP binding) and the dissociation constant (KL) for isoproterenol was established from competition curves. The effect of halothane 1%, in an air oxygen mixture (oxygen fraction: 0.3) administered by tonometry during ligand membrane incubation, on beta-adrenergic receptor, was compared to that of control experiments not exposed to halothane. Halothane produced a moderate but significant decrease of Bmax (−10%) and a significant increase in non-specific binding (+30%), while K_D, IC_{50}, and KL were unchanged. The authors conclude that halothane, *in vitro*, decreases beta-adrenergic receptor density. This effect could be mediated by an alteration of the receptor in the membrane due to action of halothane on the lipid phase of the membrane. (Key words: Anesthetic, volatile: halothane. Sympathetic nervous system: beta-receptor; ligand.)

HALOTHANE HAS BEEN SHOWN to markedly alter the function of sympathetic nervous system at different levels. It reduces preganglionic sympathetic activity[1] and ganglionic transmission.[2,3] It decreases plasma norepinephrine and epinephrine concentration[4] after a transient elevation during the first stages of anesthesia.[5] Alteration in intracellular processing of sympathetic activation, in target organs, has been also reported to occur in the presence of halothane.[6,7]

However, changes in function of the adrenergic receptors located in the membrane could also be involved in the cardiovascular effects of halothane, since halogenated anesthetics modify the physical properties of membrane.[8,9] It has been shown in a variety of circumstances that cellular response to catecholamines are altered when density or affinity of beta-adrenoceptors are lowered.[10–13] Indirect evaluations of the effect of halothane on adrenergic receptors have been performed showing an enhancement[14] or a depression,[15] but interpretation of these indirect data are difficult. A direct evaluation of the effect of halothane on beta-adrenergic receptor by radioligand binding showed no significant change.[16] The present study was designed to assess, with a highly specific ligand,[17] the effects of halothane on human beta-adrenergic receptors located in lymphocyte membranes. These changes may reflect[10,13,18] alterations in beta-adrenergic receptors located elsewhere in the body.

Materials and Methods

Blood samples (140 ml) were drawn by venipuncture from seven healthy volunteers aged 26–35 yr (five men, two women). All were normotensive and drug free, and none had evidence of any abnormality on routine questioning.

MEMBRANE PREPARATION

The membrane preparation was obtained using a modification of the method described by Feldman *et al.*[12] Heparinized whole blood was diluted with phosphate buffered saline (PBS). Then, the lymphocytes were isolated using a Ficoll-Hypaque density gradient by centrifugation (600 g for 20 min). The lymphocyte band was harvested by aspiration and resuspended in PBS. After two washings and additional centrifugations, lymphocytes were resuspended in ice-cold deionized water and broken up by ultrasonication. Aliquots were then centrifugated at 40.000 g for 20 min at 4° C. The pellet was resuspended in a conservative buffer.[12] Samples were frozen in liquid nitrogen until used for radioligand studies which were performed within 2 weeks.

BINDING ASSAYS

Beta receptor binding studies were performed according to the method of Feldman *et al.*[12] modified.

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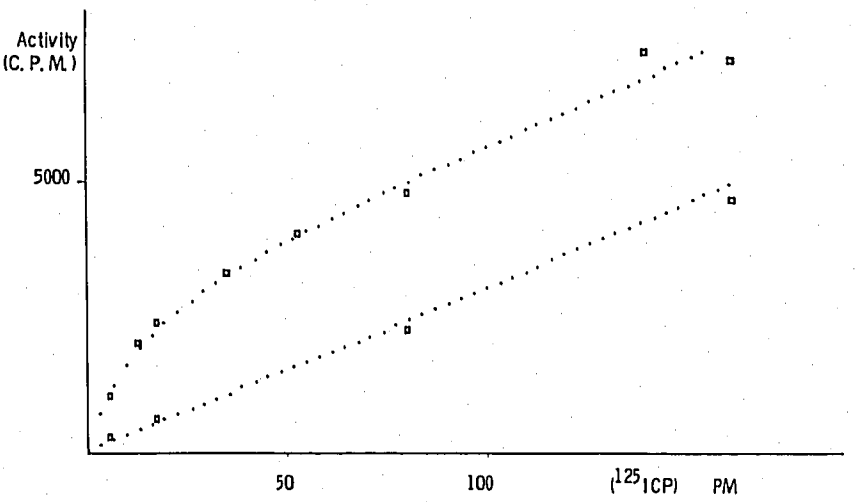

FIG. 1. Binding of ^{125}ICP to membranes of lymphocytes as function of increasing ^{125}ICP concentrations. Specific binding of ^{125}ICP was obtained by computerized fitting from total binding (upper curve, at eight concentrations) and non-specific binding in presence of 0.1 mM of isoproterenol (lower curve). Ordinate: ^{125}ICP bound in counts per minute (cpm). Abcissa: ^{125}ICP concentration in the medium.

(−)^{125}I-iodocyanopindolol (^{135}ICP) (Amersham Corp., 2200 Ci/mM) was used as radioligand. An aliquot (0,1 ml) of the membrane suspension containing 20–150 μg protein[19] was incubated, in incubation buffer,[12] with ^{125}ICP in a final volume of 250 μl. Incubations were carried out for 60 min at 37° C. For each membrane preparation, samples were shared in two groups, halothane and control. In the halothane group, a gas mixture of 1% halothane (measured by an infrared analyser, Cosma, France) in an O_2 fraction of 0.3 (N_2-O_2 mixture) was blown over suspension in all tubes of the group using a distribution system. In the control group, the gas mixture contained no halothane. The reaction was terminated by addition of 10 ml of a stopping buffer[12] followed by rapid filtration through Whatman GF/C filters. Each filter was then washed by an additional 5 ml of buffer. Radioactivity retained on the filter was determined in a gamma counter.

CALCULATION OF BINDING DATA

The density of beta adrenoreceptor in membranes was determined in saturation experiments. The amount of bound ^{125}ICP was measured at eight different concentrations ranging from 5 to 166 pM in each assay. Non-specific binding of ^{125}ICP was defined as radioactivity bound to membranes which is not displaced by 0.1 mM of (−) isoproterenol. Specific binding of ^{125}ICP is defined as total radioactivity minus non-specific binding. It is saturable at a value which represents the maximal number of binding sites (Bmax) (expressed in fm per mg of protein). The equilibrium dissociation constant for ^{125}ICP (K_D, in pM) represents the concentration of ^{125}ICP that half maximally occupies the receptors. Changes in K_D inversely relate to affinity of receptors. A typical saturation curve is represented in figure 1. Values of Bmax, K_D, and non-specific binding (in percent of total activity) were determined from experimental data by using non-linear curve fitting according to the law of action mass.[20]

Receptor affinity for the agonist was derived from the data of competition experiments (fig. 2). The binding of ^{125}ICP (at 41 pM in the buffer) was determined at 13 concentrations of the competing agent ((−) isoproterenol) ranging from 100 μM to 1 nM with and without the presence of 0.1 nM 5 guanylyl imidodiphosphate (Gpp (NH)p). It has been shown that beta-adrenergic receptors can be at two interconvertible affinity states, high and low, for the agonist.[21] Gpp has been shown to mediate transition of the entire receptor population to a low affinity state for the agonist.[12] Thus, the dissociation constant of isoproterenol obtained in the presence of Gpp correspond to the dissociation constant for the low affinity state, while, in the absence of GPP, the two affinity states are possible. Data from competition curves were analyzed by a non-linear curve fitting computerized procedure assuming one or two affinity state models, according to the presence or absence of Gpp, to determine the dissociation constant of the agonist for the high (KH) and low (KL) (nM) affinity states.[21] Affinity for isoproterenol was also evaluated by the calculation of the concentration of isoproterenol inhibiting 50% of binding (IC 50) (nM). IC 50 was calculated by logit transformation of competition curve, as previously reported.[21]

Results were expressed as mean ± SEM. The two groups were compared using the Wilcoxon test for paired data. A $P < 0.05$ was considered as significant.

Results

Binding of (−)^{125}I-iodocyanopindolol was saturable (Bmax = 39,1 ± 4,8 fm/mg of protein) and of high affinity (K_D = 6,1 ± 1,2 pM). The values of Bmax, K_D, and non-specific binding of the control set were within the normal range as judged from previous works using

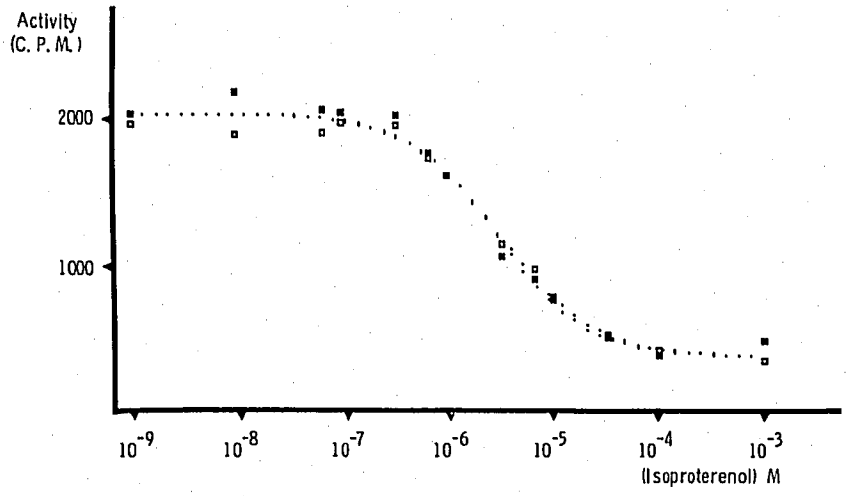

FIG. 2. Displacement of ^{125}ICP binding by (−)isoproterenol in typical competition curves. Inhibition of binding was performed in the absence (■) and presence (□) of Gpp (10^{-4} M). Bound ^{125}ICP is expressed in counts per minute. IC 50 represents the concentration of isoproterenol which displaces 50% of the ligand bound.

this ligand.[13,17] Halothane induced a significant decrease of beta receptor density (−10%) and a significant increase in non-specific binding (+30%), while the dissociation constant was unchanged (table 1, fig. 3). In all cases, fitting of competition curves showed that the best fit was obtained by assuming a one affinity state model with or without halothane. Halothane did not significantly alter the affinity of receptor for isoproterenol, since IC 50 and KL did not change (table 1).

Discussion

This study shows that a clinically relevant concentration of halothane *in vitro* induces a small but significant decrease of beta-receptor density in human lymphocytes without alteration of affinity for the beta-adrenergic antagonist. Study of beta-adrenergic receptor function can be performed by using ligand binding. Computerized fitting of the data allows determination of several parameters characterizing the properties of beta-adrenergic receptors. Interpretation of the affinity states for agonist is difficult, since we did not observe the high affinity state for the agonist in this study with this ligand, in contrast to other experiments.[22] But no difference in affinity was observed with halothane. Lymphocytes are commonly used as a model to evaluate beta-receptor function, as several studies have shown that changes in lymphocyte beta-adrenoreceptor function reflect alterations of beta-receptors located elsewhere in the body.[10,13,18] Modification of beta-adrenergic receptor function has been reported to occur in several situations. The most common mechanisms include down or up regulation of density and/or affinity of receptor in response to an increase or decrease of adrenergic activity.[10,12,18] This mechanism will not be considered in this discussion, since this study was performed *in vitro*. Thus, a direct action of halothane on the receptor itself or on the receptor ligand interaction is likely. This effect could be mediated by changes in the physical properties of the membrane which have been reported to occur with halothane. Indeed, several reports have shown that halothane induced a modification of the membrane after fixation into its lipid phase.[8,9] Thus, an alteration of the receptors into membrane sec-

TABLE 1. Alteration of Density and Affinity of Beta-adrenoceptor by Halothane

	Control	Halothane
Bmax (fm/mg protein)	39.1 ± 4.8	35.1 ± 3.7*
K_D (pM)	6.1 ± 1.2	6.6 ± 0.9
Non-specific binding	0.031 ± 0.002	0.040 ± 0.003*
IC_{50} (nM)	3241 ± 927	3616 ± 892
KL (nM)	482 ± 144	587 ± 175

* $P < 0.05$ *versus* control.

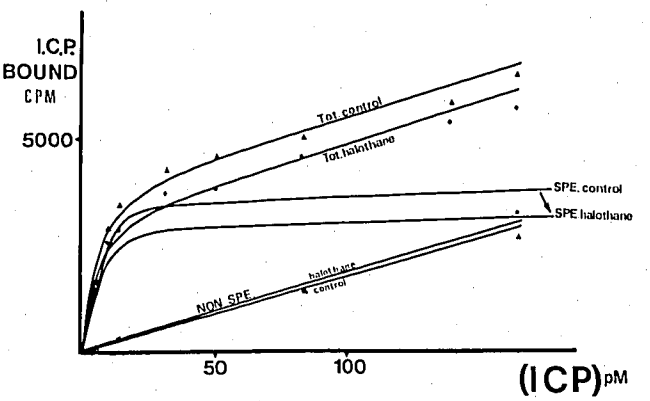

FIG. 3. A typical curves showing the effect of halothane during a saturation experiment. Total (Tot) binding, non-specific (non spe) binding and specific (spe) binding without halothane (control) and with halothane (halothane) are represented on this figure. Ordinate: ^{125}ICP bound in counts per minute. Abcissa: free ^{125}ICP concentration.

ondary to an increase in the thickness of the membrane could be a suggested mechanism. This mechanism is likely, since the affinity of the receptor was unchanged and, consequently, a direct modification of the physical properties of the receptor by halothane could be excluded. Whatever the exact mechanism involved in these changes, our findings demonstrate that the number of beta-adrenergic receptors is diminished when halothane is present in the preparation. Although the exact concentration of halothane could not have been measured in the membrane of lymphocytes, it can be assumed, however, that equilibrium of halothane with the medium containing halothane was obtained during the time of incubation.

In a previous investigation using a myocardial canine membrane preparation. Bernstein *et al.* found no significant change in beta-adrenoreceptor density, affinity, or non-specific binding in presence of halothane.[16] Although a different ligand, ^{3}H-dihydro-alprenolol, with a lower specific activity and a lower affinity for betareceptor, was used in that study, the density of receptor was also diminished (10%), but this alteration was not significant. By contrast, no change in non-specific activity was observed. Therefore, the difference between the findings from Bernstein *et al.* and our study may be only due to the different ligand used.

The physiologic implications of this study, in terms of the decrease in cellular responses which might be expected to result from a 10% reduction in the available receptors, may be discussed. A reduction in receptor number may mediate either a decrease in maximum tissue response or a change in sensitivity to the agonist (response at a higher concentration), depending of the presence of "spare receptors."[23] When no spare receptors are present, as in heart failure,[11] a full biological response requires occupancy of all the available receptors. Thus a decreased maximal response can be expected to result from a reduction in density of receptors.[23] A recent study showed that, in patients undergoing cardiac surgery, the inotropic response to isoproterenol is linearly related to beta-adrenoreceptor density in myocardium.[13] The consequence of the reduction in receptor number will be to decrease tissue sensitivity to agonist.[23] In fact, the full response could be regained by increasing beta-adrenergic receptor occupancy with a higher concentration of agonist. However, halothane also reduces sympathetic activity.[1] Thus, the observed changes could account, at least in part, for some pharmacological effects of halothane, *i.e.*, the decrease of myocardial contractility, particularly in patients with heart disease, or the anesthetic action of halothane by reducing adrenergic transmission in the central nervous system. It is likely that halothane also acts on alpha-adrenergic receptors, since it induces a hypo-responsiveness to alpha-adrenergic stimulation in rabbits.[11] It can be concluded that the method used in this study allows analysis of the *in vitro* effects of halothane on beta-adrenergic receptors. The findings may explain some of pharmacologic actions of halothane, but further *in vivo* studies are required to support this view. In addition, other experiments at higher concentrations would be useful to determine if a dose-effect relationship exists.

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References

1. Garfield JM, Alper MN, Gillis RA, Flacke W: A pharmacological analysis of ganglionic action of some general anesthetics. ANESTHESIOLOGY 29:79–92, 1968
2. Skovsted P, Price ML, Price HL: The effect of halothane on arterial pressure, preganglionic sympathetic activity and barostatic reflexes. ANESTHESIOLOGY 31:507–512, 1969
3. Christ D: Effects of halothane on ganglionic discharges. J Pharmacol Exp Ther 200:336–342, 1977
4. Marty J, Couture J, De Champlain J: Plasma sulfate conjugated catecholamines during anesthesia. ANESTHESIOLOGY 59:162, 1983
5. Joyce JT, Roizen MF, Gerson JI: Induction of anesthesia with halothane increases plasma norepinephrine concentrations. ANESTHESIOLOGY 56:286–290, 1982
6. Gangat Y, Vulliemoz Y, Verosky M, Danilo P, Bernstein K, Triner L: Action of halothane on myocardial adenylate cyclase of rat and cat. Proc Soc Exp Biol Med 160:154–159, 1979
7. Yang JC, Triner L, Vulliemoz Y, Verosky M, Ngai SH: Effects of halothane on the cyclic adenosine 3'5' monophosphate (cyclic AMP) system in rat uterine muscle. ANESTHESIOLOGY 38:244–250, 1973
8. Trudell JR, Hubbell WL: Localization of molecular halothane in phospholipid bilayer model nerve membranes. ANESTHESIOLOGY 44:202–206, 1976
9. Rosenberg PH, Jannson S, Gripenberg J: Effects of halothane, thiopental and lidocaine on fluidity of synaptic membranes and artificial phospholipid membranes. ANESTHESIOLOGY 46:322–326, 1977
10. Fraser J, Nadeau J, Robertson D, Wood AJJ: Regulation of human leukocyte betareceptors by endogenous catecholamines. Relationship of leukocyte beta-receptor density to the cardiac sensitivity of isoproterenol. J Clin Invest 67:1777–1784, 1981
11. Bristow MR, Ginsburg R, Minobe W, Cubicciotti RS, Sageman WS, Lurie K, Billingham ME, Harrison DC, Stinson EB: Decreased catecholamine sensitivity and beta-adrernergic receptor density in failing human hearts. N Engl J Med 307:205–211, 1982
12. Feldman RD, Limbird LE, Nadeau J, Fitzgerald GA, Robertson D, Wood AJJ: Dynamic regulation of leukocyte beta-adrenergic receptor agonist interactions by physiological changes in circulating catecholamines. J Clin Invest 72:164–170, 1983
13. Brodde OE, Kretsch R, Ikezono K, Zerkowski HR, Reidemeister JC: Human beta-adrenoreceptors: Relation of myocardial and lymphocyte beta-adrenoreceptor density. Science 231:1584–1585, 1986
14. Price HL, Skovsted P, Pauca AL, Cooperman LH: Evidence for

beta-receptor activation produced by halothane in normal man. ANESTHESIOLOGY 32:389–395, 1970

15. Spiss CK, Smith CM, Tsujimoto G, Hoffman BB, Maze M: Prolonged hyporesponsiveness of vascular smooth muscle contraction after halothane anesthesia in rabbits. Anesth Analg 64:1–6, 1985
16. Bernstein KJ, Gangat Y, Verosky BA, Vulliemoz Y, Triner L: Halothane effect on beta-adrenergic receptors in canine myocardium. Anesth Analg 60:401–405, 1981
17. Brodde OE, Engel G, Hoyer D, Bock KD, Weber F: The beta adrenergic receptor in human lymphocytes. Subclassification by the use of a new radioligand, ^{125}Iodocyanopindolol. Life Sci 29:2189–2198, 1981
18. Aarons RD, Molinoff PB: Changes in the density of beta-adrenergic receptors in rat lymphocytes, heart and lung after chronic treatment with propranolol. J Pharmacol Exp Ther 221:439–443, 1982
19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275, 1951
20. Munson PJ, Rodbard D: Ligand: A versatile computerized approach for characterization of ligand binding system. Anal Biochem 107:220–239, 1980
21. Kent RS, DeLean AL, Lefkowitz RJ: Quantitative analysis of beta-adrenergic receptor interactions: Resolution of high and low affinity states of the receptor by computer modelling of ligand binding data. Mol Pharmacol 17:14–23, 1980
22. Brodde OE, Karad K, Zerkowski HR, Rohm N, Reidemeister JC: Coexistence of $beta_1$ and $beta_2$ adrenoreceptors in human right atrium: Direct identification by (±) ^{125}I iodocyanopindolol binding. Circ Res 53:752–758, 1983
23. Lefkowitz RJ: Direct binding studies of adrenergic receptors: biochemical, physiologic and clinical implications. Ann Intern Med 91:450–458, 1979