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Influence of halothane on the interactions of serotonin$_{1A}$ and adenosine A_1 receptors with G proteins in rat brain membranes

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Certain volatile general anesthetics depress synaptic transmission by interfering with receptor–G protein interactions [1]. Halothane and other volatile anesthetics disrupt signal transduction by two neurotransmitter receptors, the muscarinic acetylcholine and α_2-adrenergic receptors, whose synaptic actions are mediated by pertussis toxin-sensitive G proteins [1–5]. This disruption is seen as a depression of the guanine nucleotide sensitivity of agonist binding, as well as an interference with the inhibitory control of these receptors over adenylate cyclase activity. In contrast, halothane does not affect the stimulation of adenylate cyclase activity by β-adrenergic receptors, an action which is mediated by the stimulatory, pertussis toxin-insensitive G_s protein [6].

These findings raise the possibility that volatile anesthetic disruption of receptor–G protein coupling is restricted to the family of neurotransmitter receptors which preferentially couple to G proteins which serve as substrates for ADP-ribosylation catalyzed by pertussis toxin, particularly G_i and G_o. To examine the validity of this suggestion, we examined the effects of halothane on adenosine A_1 and serotonin$_{1A}$ (5-HT$_{1A}$) receptors. A_1 and 5-HT$_{1A}$ receptor agonists inhibit adenylate cyclase activity through G_i protein activation [7, 8]. Moreover, the involvement of A_1 and 5-HT$_{1A}$ receptors in the regulation of sleep and analgesia makes them reasonable potential targets of anesthetic action [9–11].

Materials and Methods

Rats were decapitated and brain tissue was removed and homogenized in 15 mL of 50 mM Tris–HCl buffer, pH 7.4, containing 2 mM $MgCl_2$ and 1 mM dithiothreitol. The homogenate was centrifuged at 15,000 *g* for 20 min. The supernatant was discarded and the pellet washed twice by resuspension and centrifugation under the same conditions. The final pellet was suspended in buffer at a final protein concentration of 1 mg/mL. The G protein interactions of muscarinic and α_2-adrenergic receptors in neural membranes prepared in 50 mM Tris–HCl/2 mM $MgCl_2$ buffer were disrupted by anesthetics (e.g. [4]). Moreover, inclusion of 1 mM dithiothreitol in the buffer (as in the present experiments) did not alter these actions of halothane (e.g. [2]). Hippocampal membranes were used for 5-HT$_{1A}$ binding assays, while forebrain membranes were used for A_1 binding assays. Membranes used in [^{3}H]-cyclohexyladenosine ([^{3}H]CHA) binding assays were incubated for 30 min at room temperature with adenosine deaminase (0.2 unit/mL) to remove endogenous adenosine.

Assays were initiated by addition of the membranes to a binding medium containing either 1 nM 8-hydroxy-dipropylaminotetralin ([^{3}H]8-OH-DPAT; DuPont-NEN; 142.9 Ci/mmol) or 1 nM cyclohexyladenosine ([^{3}H]CHA; DuPont-NEN; 34.4 Ci/mmol). Incubations were carried out at 37° until binding equilibrium was reached (10 min, [^{3}H]8-OH-DPAT; 60 min, [^{3}H]CHA). Guanyl-5'-imidodiphosphate (Gpp(NH)p; Sigma), a stable analogue of GTP, was included in some assays. Non-specific binding was determined in the presence of 100 μM 5-HT or 1 mM theophylline in the 5-HT$_{1A}$ and A_1 receptor binding assays, respectively. Specific binding represented 80–90% of the total binding of either probe at 1 nM. Membranes were collected by vacuum filtration on glass fiber filters, and the radioactivity content of the filters was determined by liquid scintillation counting.

Under these conditions, the density of [^{3}H]8-OH-DPAT binding sites was 258 ± 35 fmol/mg membrane protein, while the dissociation constant was 1.1 ± 0.2 nM (means ± SD, N = 4). [^{3}H]CHA labeled 222 ± 19 fmol binding sites/mg membrane protein with a dissociation constant of 39 ± 0.5 nM (means ± SD, N = 4).

Anesthetics were added to assay tubes as aliquots from stock buffers. Anesthetic concentrations were verified by gas–liquid chromatographic analysis of 1-μL aliquots obtained from sham reaction tubes.

Results

Halothane inhibited the binding of [^{3}H]8-OH-DPAT to 5-HT$_{1A}$ receptors in hippocampal membranes by up to 23% at 1.8 mM (Fig. 1A). Inclusion of the guanine nucleotide, Gpp(NH)p, during the incubation had little effect on the inhibition by halothane.

In agreement with previous reports [12, 13], Gpp(NH)p

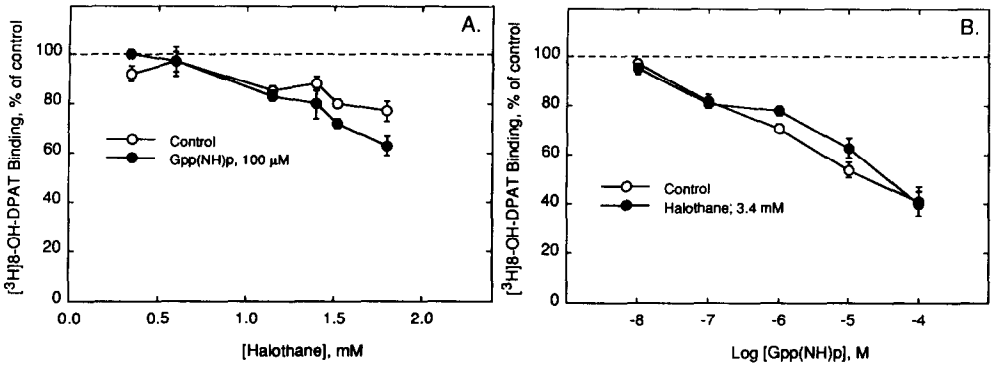

Fig. 1. Influence of halothane on agonist binding to 5-HT_{1A} receptors in rat hippocampal membranes. (A) The specific binding of 1 nM [^{3}H]8-OH-DPAT was measured in the presence of the indicated concentrations of halothane. Binding, expressed as a percent of control binding in the absence of halothane, was measured in the absence (○) and presence (●) of 100 μM Gpp(NH)p. In the absence of halothane, [^{3}H]8-OH-DPAT binding was 103 ± 6 and 58 ± 7 fmol/mg protein (means ± SD, N = 4) in the absence and presence of 100 μM Gpp(NH)p, respectively. (B) The specific binding of 1 nM [^{3}H]-8-OH-DPAT was measured in the presence of the concentration of Gpp(NH)p indicated on the abscissa in the absence (○) and presence (●) of 3.4 mM halothane. Binding is expressed as a percent of control binding measured in the absence of Gpp(NH)p. Two-way ANOVA for repeated measures indicated no alteration in the sensitivity of [^{3}H]8-OH-DPAT binding to Gpp(NH)p in the presence of 3.4 mM halothane. In the absence of Gpp(NH)p, [^{3}H]8-OH-DPAT binding was 108 ± 9 and 61 ± 5 fmol/mg protein (means ± SD, N = 4) in the absence and presence of 3.4 mM halothane, respectively. Values are the means ± SD from 4 experiments.

decreased the binding of the agonist [^{3}H]8-OH-DPAT (Fig. 1B). Depression of high-affinity agonist binding by guanine nucleotides is thought to reflect a dissociation of receptor and transducer G protein, which leaves the receptor in a state characterized by low affinity for agonists. [^{3}H]8-OH-DPAT (1 nM) binding was decreased 60% by 100 μM Gpp(NH)p. The ability of Gpp(NH)p to inhibit [^{3}H]8-OH-DPAT binding was not affected by exposure of the membrane to a high concentration (3.4 mM) of halothane (Fig. 1B).

The binding of [^{3}H]CHA to adenosine A_1 receptors in membranes from rat forebrain was affected only slightly by halothane (20% inhibition at 2 mM halothane; Fig. 2A). Gpp(NH)p (100 μM) did not affect this inhibition. Gpp(NH)p inhibited [^{3}H]CHA binding to A_1 receptors by up to 66% at 100 μM (Fig. 2B). Halothane (1.9 mM) did not affect this inhibition.

Discussion

The current experiments examined the effects of halothane on the guanine nucleotide sensitivity of agonist binding to adenosine A_1 and 5-HT_{1A} receptors. Because clinically relevant concentrations of halothane (e.g. 0.4 to 0.8 mM) disrupt G protein–receptor interactions in two other receptor systems that inhibit adenylate cyclase activity via G_i (i.e. muscarinic m2 acetylcholine and α_2-adrenergic receptors [4–6]), a similar effect of the anesthetic on 5-HT_{1A} and A_1 receptors was anticipated. The results revealed a modest inhibition of high-affinity agonist binding at high concentrations of halothane (i.e. high relative to anticipated therapeutic concentrations—see below) and no interference with receptor–G protein coupling.

Equilibration of 50 mM Tris–HCl buffer with 1 MAC halothane at 37° [1% (v/v) halothane for rats; MAC is the minimum alveolar concentration of anesthetic gas which produces surgical anesthesia in one half of the tested population] resulted in buffer halothane concentrations of 0.42 mM (data not shown). Thus, the concentrations of halothane required to influence agonist binding to either 5-HT_{1A} or adenosine A_1 receptors in this study were considerably greater than those anticipated during clinical use.

A generalized depression of synaptic transmission underlies the elaboration and maintenance of the anesthetic state [14]. It is also likely that the primary effect of general anesthetics is an alteration in a function of a specific protein(s) involved in synaptic transmission [15, 16]. In view of their strategic location and critical functions, both receptors and G proteins are logical candidates for critical sites in anesthetic action. It is clear from previous results [1–5] that various volatile anesthetics, at clinically-relevant concentrations, disrupt receptor–G protein interactions at certain neurotransmitter synapses. However, the present results demonstrate that the disruptive influence of volatile anesthetics on receptor–G protein interactions is not generalized to all receptors, or even to all receptors that mediate inhibition of adenylate cyclase via G_i. Thus, only a subset of receptors may be involved in anesthetic depression of synaptic transmission by this mechanism.

In summary, the influence of halothane on the interactions of 5-HT_{1A} and adenosine A_1 receptors with G proteins was determined by monitoring the guanine nucleotide sensitivity of agonist binding to these receptors. Halothane inhibited the binding of radiolabeled agonists to 5-HT_{1A} and adenosine A_1 receptors by up to 30%, but only at concentrations considerably greater than those necessary for the maintenance of the anesthetic state. The sensitivity of high-affinity agonist binding to a guanine nucleotide (guanylyl-5′-imidodiphosphate) was not affected by halothane, indicating no disruption of receptor–G protein coupling. Thus, it appears that the ability of halothane to disrupt receptor-mediated signal transduction by interference with receptor–G protein interactions is receptor specific.

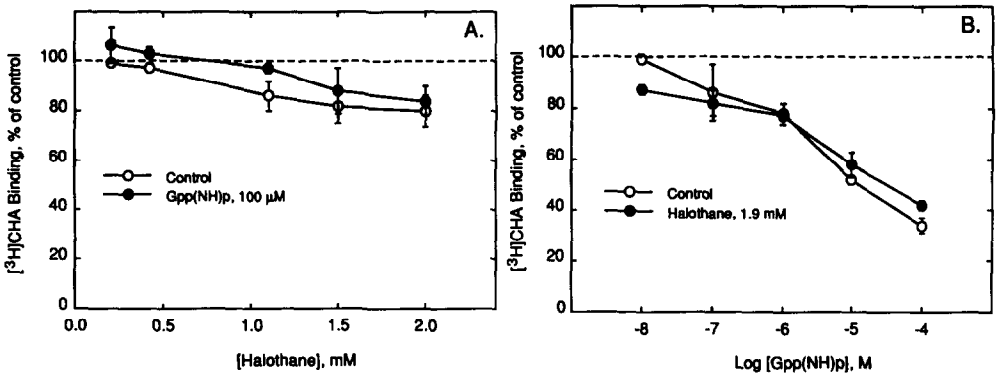

Fig. 2. Influence of halothane on agonist binding to A_1 receptors in rat forebrain membranes. (A) The specific binding of 1 nM [^{3}H]CHA was measured in the presence of the indicated concentrations of halothane. Binding, expressed as a percent of control binding in the absence of halothane, was measured in the absence (○) and presence (●) of 100 μM Gpp(NH)p. In the absence of halothane, [^{3}H]CHA binding was 50 ± 8 and 31 ± 4 fmol/mg protein (means ± SD, N = 4) in the absence and presence of 100 μM Gpp(NH)p, respectively. (B) The specific binding of 1 nM [^{3}H]CHA was measured in the presence of the concentration of Gpp(NH)p indicated on the abscissa in the absence (○) and presence (●) of 1.9 mM halothane. Binding is expressed as a percent of control binding measured in the absence of Gpp(NH)p. Two-way ANOVA for repeated measures indicated no alteration in the sensitivity of [^{3}H]CHA binding to Gpp(NH)p in the presence of 1.9 mM halothane. In the absence of Gpp(NH)p, [^{3}H]CHA binding was 52 ± 6 and 46 ± 3 fmol/mg protein (means ± SD, N = 4) in the absence and presence of 1.9 mM halothane, respectively. Values are the means ± SD from 4 experiments.

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Variability of sarin-induced hypothermia in mice: investigation into incidence and mechanism

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Sarin (isopropyl methylphosphonofluoridate) is a potent acetylcholinesterase inhibitor. The signs of poisoning (e.g. salivation, lacrimation, diarrhea, and tremors) are typical of cholinergic overstimulation. In rodents, a transient hypothermia is evident following administration of an organophosphate anticholinesterase [1–4]. It was noted in my laboratory that the incidence of sarin-induced hypothermia was variable following administration of a sublethal dose of sarin to mice, i.e. there were mice which displayed hypothermia (responders) and those which did not display hypothermia (non-responders). The present study documents the incidence of non-responders following sarin administration and investigates the mechanism behind the variable response of sarin-induced hypothermia in mice.

Male CD-1 mice (25–30 g) obtained from Charles River Canada Ltd., St. Constant, Quebec, were used in this study. The animals were kept in the vivarium at Defence Research Establishment Suffield for at least 1 week, following their arrival, prior to experimentation. The animals were allowed access to food and water *ad lib*. The room temperature was 21–22°.

For the determination of the temporal response following sarin administration, core temperature was monitored using telemetry [2]. The mice were allowed to recover for 1 week following implantation of the telemetry transmitter, prior to use in an experimental situation, at which time the telemetry transmitters were activated, the mice were placed in individual cages, and the core temperature was monitored. Typically, the first three data points established a control baseline. Sarin was administered immediately after the acquisition of the third data point. The data were acquired at 30-min intervals for a total of 720 min. The entire time period, including the control interval, was then used in the calculation of the mean minimum temperature and the area under the curve (AUC).

Brain tissue (hypothalamus, hippocampus, and cortex) was isolated and homogenized using a glass–teflon homogenizer in 0.01 M Tris buffer, pH 7.4, containing 1 M NaCl, 0.05 M $MgCl_2$ and Triton X-100 (1%). The homogenate was centrifuged at 5000 *g* for 20 min, and the acetylcholinesterase activity was determined in a microplate assay using the procedure of Ellman *et al.* [5]. For determination of plasma carboxylesterase activity, blood was sampled from the orbital sinus using heparinized hematocrit tubes. These were centrifuged, to separate the plasma, and the carboxylesterase activity was determined by a spectrophotometric assay using *p*-nitrophenylacetate as the substrate [6].

Sarin was prepared at Defence Research Establishment Suffield. The 24-hr LD_{50} of the sarin used in this study was between 160 and 170 μg/kg (s.c.). Sarin was dissolved in saline prior to injection and the volume of injection was 1% of body weight. Significant differences of the means were determined by Student's *t*-test. A value of $P < 0.05$ was considered statistically significant.

In responders, the sarin-induced hypothermia was rapid in onset, reached a maximum in approximately 2 hr and returned to control levels by 10–12 hr after administration (Fig. 1). In a certain population of those mice exposed to sarin (130 μg/kg, s.c.) hypothermia did not develop. The incidence of hypothermia non-responders varied from 25 to 67% for any particular experiment with an overall value of 41.7 ± 14.6% (N = 10–12 repeated ten times for total of 112) following adminstration of sarin (130 μg/kg, s.c.). In the non-responders, the signs of poisoning appeared to be absent, i.e. there was no sign of tremors, salivation, lacrimation, hypothermia, etc. It was as though the animals were not exposed to sarin.

Acetylcholinesterase activity was determined in the hypothalamus, hippocampus and cortex 90 min after sarin administration (Table 1). Brain acetylcholinesterase activity in both responders and non-responders was severely inhibited compared to the unexposed control group. However, the acetylcholinesterase activity in the hypothalamus, hippocampus and cortex in the non-responders was significantly higher than that of the responders. From these results there appeared to be a threshold of brain acetylcholinesterase inhibition required for the appearance of hypothermia in mice, >52% in hypothalamus, > 65% in hippocampus and > 82% in cortex. Interestingly, even though there were different degrees of acetylcholinesterase inhibition in the various brain areas of non-responders, there were no overt signs of cholinergic overstimulation. Previous authors have reported that there is a critical level of brain acetylcholinesterase inhibition, in particular in the hypothalamus, which is required for hypothermia to be expressed [7, 8]. These results were confirmed in the present study following sarin poisoning in mice where hypothalamic acetylcholinesterase inhibition must be > 52% for the appearance of hypothermia.

The differences in the degree of inhibition of acetylcholinesterase in the various brain regions between