Title: THE ROLE OF PERTUSSIS TOXIN-SENSITIVE G PROTEINS IN THE ANESTHETIC ACTION OF HALOTHANE IN RATS

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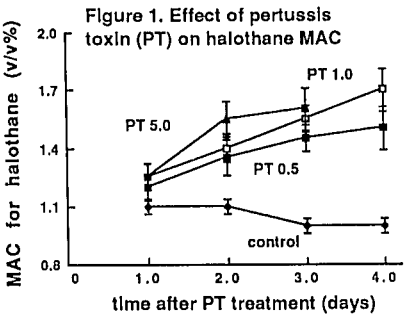
Introduction: Structurally diverse chemical agents are capable of producing anesthesia. This lack of structure-activity relationship has perpetuated the historical viewpoint that volatile anesthetics act by physically changing lipid portions of nerve membranes. Recent evidence challenges these concepts and supports the view that anesthetics act specifically on membrane proteins.[1] Among the candidates for the putative binding site of volatile anesthetics in the brain are the guanine nucleotide-binding proteins (G proteins). These G proteins are abundantly present in nerve membranes where they function as transducers of the effector mechanism for several inhibitory neurotransmitter receptors (e.g. alpha$_2$ adrenergic, A_1 adenosine, D_2 dopaminergic).[2] G proteins are inactivated by toxins produced by *b. pertussis* which provides a useful tool for studying the function of these proteins. Interestingly, the pertussis toxin (PT)-sensitive G proteins are coupled to alpha$_2$ adrenergic receptors[3] which when stimulated with potent agonists, such as MPV-1440 (MPV), mediate a response that is similar to general anesthesia.[4] This study was designed to: 1) determine the dependence of halothane anesthesia on PT-sensitive G proteins, and 2) determine the dependence of MPV's MAC-reducing action on the PT-sensitive G protein.

Methods: This study was approved by our institutional Animal Care and Use Committee. Male Sprague Dawley rats (180-220 g) were tested at the same time each day. To determine the dependence of halothane anesthesia on G proteins, PT, 0.5, 1.0 and 5.0 ug, or vehicle was administered intracerebroventricularly (icv). On the 3 successive days following treatment, the MAC for halothane was determined. To determine whether PT-sensitive G proteins are involved in the MAC-reducing effect of the alpha$_2$ adrenergic agonist MPV, the MAC for halothane was determined in PT-treated or control rats following administration of MPV, 30 ug/kg i.p. (This dose was earlier shown to be the MPV IC_{50} for reduction of halothane MAC).[4]

Each experiment was performed in duplicate using 10 rats for each treatment dose. ANOVA followed by Student's t-test with Bonferroni correction were applied to evaluate the significance of the results.

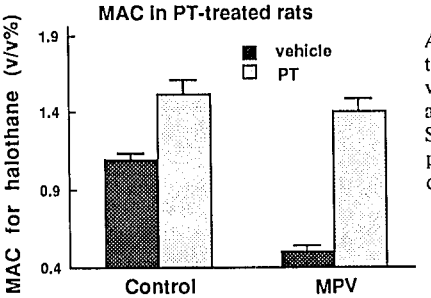
Results: PT increased halothane MAC in a dose-dependent manner to a maximum of 170% of control ($p<0.05$) (Figure 1).

Figure 1. Effect of pertussis toxin (PT) on halothane MAC



At each dose, a total of 10 mice were tested. Data are the mean and SD of experiments performed in duplicate.

Figure 2. Effect of MPV on halothane MAC in PT-treated rats



At each dose, a total of 10 mice were tested. Data are the mean and SD of experiments performed in duplicate.

PT also increased halothane MAC in a time-dependent manner (Figure 1). As expected the dose of MPV selected (30 ug/kg) decreased halothane MAC by ~50%. In PT-treated rats MPV did not affect halothane MAC compared to control (Figure 2).

Discussion: These data indicate that rats treated with icv PT require a higher halothane concentration to achieve 1.0 MAC. The decreased sensitivity to halothane suggests a mediating role for PT-sensitive G proteins in the molecular mechanism for its anesthetic action. PT-sensitive G proteins in the brain are thought to transduce responses *via* alpha$_2$ adrenergic, A_1 adenosine, D_2 dopamine, and opiate receptors.[2] Each of these receptor-effector mechanisms cause membrane hyperpolarization and decrease anesthetic requirements.[3,5] Halothane anesthesia also is characterized electrophysiologically by membrane hyperpolarization although the mechanism for this effect is not understood.[6] The PT-sensitive G proteins are present in sufficiently high concentration to accommodate the millimolar halothane concentration in the brain necessary for anesthetic action.[7] Also, these proteins are capable of transducing membrane hyperpolarization.[2,3] Our findings, that inactivation of PT-sensitive G proteins increases anesthetic requirements, suggest that G proteins are potential membrane sites at which anesthetic agents mediate their functional effects. Secondly, the MAC-reducing action of MPV (an alpha$_2$ agonist) is attenuated by PT treatment suggesting that this action of alpha$_2$ adrenergic agonists is dependent on a PT-sensitive G protein.

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