

Lack of evidence for muscarinic type 2 receptor modulation of the human nasonasal reflex

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In animal models, acetylcholine released from the vagus nerve contracts airway smooth muscle by muscarinic type 3 (M3) receptor stimulation. Acetylcholine also feeds back onto muscarinic type 2 (M2) autoreceptors, limiting further release of acetylcholine. M2 antagonists such as gallamine can block these inhibitory autoreceptors and intensify airway bronchoconstriction to vagal stimulation up to 10-fold.¹ Conversely, vagally induced bronchoconstriction can be suppressed up to 80% when M2 receptors are stimulated with pilocarpine. Interestingly, a number of inflammatory processes in animals, including allergy, viral infection, and ozone exposure, can negatively affect M2 receptor function, with resultant airway hyperresponsiveness and increased reflex bronchoconstriction.²

The role of M2 receptors in human beings is unknown. The parasympathetic nervous system, however, plays an important role in nasal glandular secretion.³ A demonstration of this is the nasonasal reflex. Challenging one nostril with antigen, cold air, capsaicin, and histamine leads to glandular stimulation in the opposite nostril. In prior studies, we demonstrated that atropine could significantly inhibit the efferent arm of the nasonasal reflex to unilateral histamine challenge.⁴ We therefore studied the effect of M2 autoreceptor modulation on the parasympathetic efferent arm of the nasonasal histamine-induced reflex. We hypothesized that inhibition of the M2 receptor on the contralateral side of histamine challenge by gallamine would lead to an increase in the parasympathetically mediated reflex response, whereas stimulation of the M2 receptor by pilocarpine would lead to a decreased glandular response.

Abbreviations used

M2: Muscarinic type 2
M3: Muscarinic type 3

METHODS

Ten normal subjects were included in this study and were specifically chosen in an attempt to avoid the potential loss of M2 receptor through ongoing allergic inflammation. The institutional review board of the University of Chicago approved the study, and written informed consent was obtained from each subject.

This was a randomized, double blind, placebo-controlled, 3-way crossover design comparing the effects of increasing doses of gallamine (1, 10, 100, and 1000 ng), pilocarpine (5, 50, 500, and 5000 µg), and placebo on the contralateral nasal response to a unilateral histamine challenge. Drugs were administered from a metered dose inhaler to the side opposite that of histamine stimulation. The response to histamine was measured by obtaining the weight of generated nasal secretions on the side of stimulation (ipsilateral) and in the opposite (contralateral) nostril as previously described.³ Visits were separated by 7 ± 1 days to eliminate any carryover effect of the challenges or drugs.

At each visit, baseline secretion weights were obtained. Subjects then received placebo delivered to the contralateral nostril. Three minutes later, a challenge with 0.1 mg of histamine was performed in the ipsilateral nostril, and the resultant secretions were collected on both sides on filter paper disks placed for 30 seconds. Following the same pattern at 12-minute intervals, subjects were treated with gallamine, pilocarpine, or placebo and then challenged with 0.1 mg of histamine after each dose.

Because the data were normally distributed, they were analyzed by use of parametric statistics and are reported as mean ± SEM. Repeated-measures ANOVA was performed, followed by post hoc analysis by paired *t* test.

RESULTS

Compared with baseline, there was a significant increase in the ipsilateral and contralateral histamine-induced secretion weights after initial placebo treatment for all visits, indicating a nasonasal reflex (Fig 1). Contralateral (reflex) secretion weights after histamine challenge showed no change from the initial placebo treatment at any time point in either the placebo treatment arm (ANOVA, *P* = .96) or the gallamine arm (ANOVA, *P* = .24). There was, however, a significant dose-dependent increase in the reflex response to histamine when

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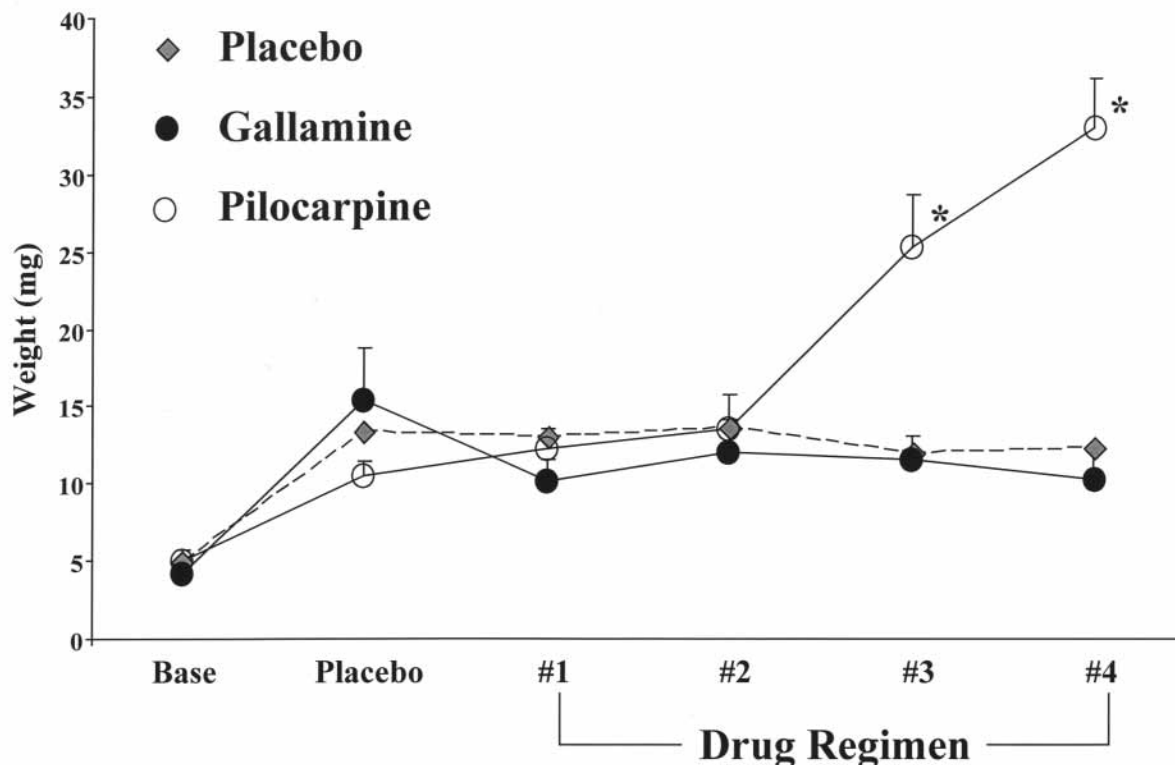


FIG 1. Contralateral secretory response to histamine. Secretion weights are shown on the *y-axis*, and the challenge protocol is shown on the *x-axis*. Base, Baseline measurement; Placebo, histamine-induced secretion weights after initial pretreatment with saline solution; #1, #2, #3, and #4, histamine-induced secretion weights after treatment with either placebo or increasing doses of gallamine or pilocarpine. * $P < .05$ versus the response obtained after initial placebo treatment.

subjects were treated with pilocarpine (ANOVA, $P = .0001$) at doses of 500 μg and 5000 μg ($P < .05$).

DISCUSSION

The reproducible nasonasal reflex induced by histamine stimulation provides a means for testing drug effects on the efferent, parasympathetic limb. Treatment with gallamine over a 4-log dose range had no effect on the response, whereas pilocarpine at high doses caused an increased response, the opposite of that predicted for M2 receptor stimulation. The effect of pilocarpine showed that the drugs were present and active in the nasal mucosa. In data not shown, application to the nasal mucosa of pilocarpine, at the same doses used in this study, caused an increase in secretion weights on the side of application without causing a nasonasal reflex, suggesting that pilocarpine induces direct M3 stimulation. Previous experiments with the 2 lowest doses of the agents over a dose-response curve of histamine stimulation also yielded negative results (data not shown). Our

data suggest the functional absence of M2 receptors in the normal adult human nose. Our pharmacologic findings are consistent with the work of Okayama et al,⁵ who were unable to demonstrate an M2 receptor by autoradiography. These findings could be related to differences in the species or to the fact that prior infections, or other inflammatory processes, may have destroyed the M2 receptor.

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