

Cutaneous responses to anticholinergics: Evidence for muscarinic receptor subtype participation

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We conducted a series of experiments to determine if anticholinergics induce immediate cutaneous wheal-and-flare responses in normal volunteers. We performed intradermal skin testing in seven healthy volunteers (three atopic and four nonatopic) with 20 nmol of atropine. All subjects had an immediate wheal-and-flare response to atropine. To determine if this cutaneous response was due to anticholinergics in general, skin testing was also performed to scopolamine and ipratropium. These agents also produced immediate wheal-and-flare responses in all subjects, but they less potent than atropine. Pretreatment with antihistamines equivalently inhibited wheal-and-flare responses to both histamine and atropine, indicating a possible mast cell role. The potential role of M1, M2, and M3 muscarinic receptor subtypes was evaluated by use of the selective antagonists, pirenzepine (M1), 11-[[2-[(diethylamino)methyl]-1-piperidinyl]-acetyl]-5,11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepine-6-one (AFDX-116) (M2), and 4-diphenylacetoxy-N-methylpiperidine (4-DAMP) (M3). Cutaneous wheal responses induced by pirenzepine and 4-DAMP were relatively equivalent and larger than responses induced by AFDX-116 at doses <200 nmol. At 200 nmol, all three muscarinic receptor subtype antagonists induced equivalent wheal formation. We then compared the cutaneous wheal responses to these specific muscarinic receptor antagonists based on their relative affinity for their respective muscarinic receptor subtype. This comparison suggested that M1 or M2, but not M3, muscarinic receptor subtypes may be important in anticholinergic-induced cutaneous wheal-and-flare responses. We propose that there may be an M1 or M2 muscarinic autoreceptor that inhibits the release of acetylcholine and other neurotransmitters. Anticholinergic-induced cutaneous wheal-and-flare responses may then result from inhibition of this autoreceptor, thereby allowing release of acetylcholine and other neurotransmitters leading to an immediate wheal-and-flare response. (J ALLERGY CLIN IMMUNOL 1991;87:971-6.)

We recently received a consultation requesting evaluation of a patient with possible atropine-induced anaphylaxis. A review of the literature revealed that case reports of atropine hypersensitivity are rare. We did find two case reports of possible allergic reactions to atropine with positive intradermal skin testing to atropine.^{1,2} However, neither article clearly documented atropine skin testing in control subjects. We therefore

Abbreviations used

AFDX-116: 11-[[2-[(diethylamino)methyl]-1-piperidinyl]-acetyl]-5,11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepine-6-one
4-DAMP: 4-Diphenylacetoxy-N-methylpiperidine

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designed a clinical study to further evaluate atropine-induced cutaneous responses. Our goals of the study were initially to determine skin test responsiveness to anticholinergic agents, particularly atropine, in healthy volunteers. Once positive immediate cutaneous wheal-and-flare responses were observed, we evaluated the effect of pretreatment with antihistamines to determine if mast cell degranulation played a possible role in these responses. Finally, we attempted to determine what muscarinic receptor subtypes were involved in producing immediate cutaneous

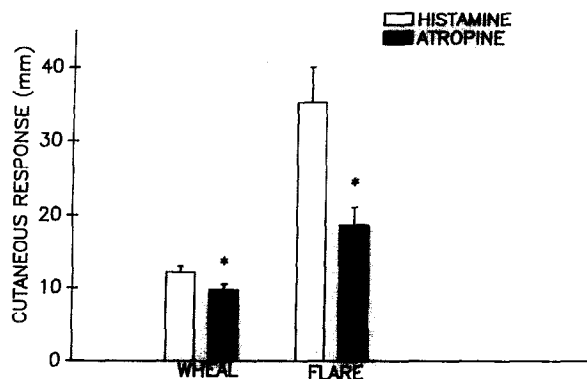


FIG. 1. Cutaneous responses to histamine and atropine. Values represent the mean wheal-and-flare responses of seven individuals 15 minutes after intradermal injection of 20 nmol of both histamine ($n = 9$) and atropine ($n = 14$); *significantly different from histamine response, $p < 0.01$.

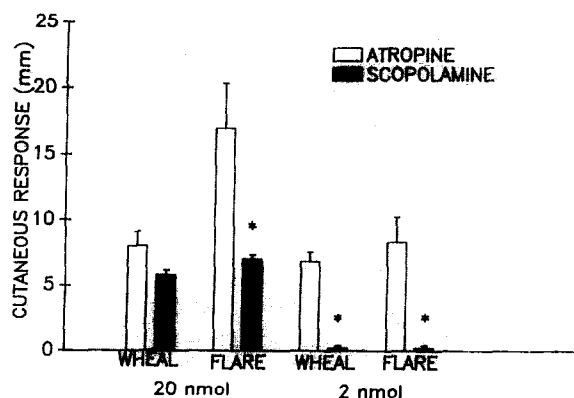


FIG. 2. Cutaneous responses to atropine and scopolamine. Values represent the mean wheal-and-flare responses of five individuals 15 minutes after intradermal injection of 20 nmol and 2 nmol of atropine and scopolamine; *significantly different from atropine, $p < 0.01$.

neous wheal-and-flare responses by skin testing with the specific muscarinic receptor subtype antagonists, pirenzepine (M1), AFDX-116 (M2), and 4-DAMP (M3).

MATERIAL AND METHODS

Skin tests

Intradermal skin testing was performed as previously described in seven healthy volunteers, including three atopic individuals with seasonal allergic rhinitis and four nonatopic individuals.^{3,4} Volunteers included three female and four male subjects with an age range from 20 to 43 years. All reagents were diluted in 0.9% saline, 0.03% human albumin, and 0.4% phenol in water (Hollister-Stier Laboratories, Spokane, Wash.) and then buffered to a pH range of 6.5 to 7.5. All skin testing was accompanied by a positive control of 20 nmol of histamine diphosphate (Eli Lilly, Indianapolis, Ind.) and a negative control of diluent alone. Test reagents used included commercial atropine sulfate (Elkins-Sinn, Inc., Cherry Hill, N. J.) and laboratory-grade atropine sulfate (Sigma Chemical Co., St. Louis, Mo.), scopolamine hydrobromide (LyphoMed, Inc., Rosemont, Ill.), ipratropium bromide, and AFDX-116 (gifts from J. Bloom, Tucson, AZ), pirenzepine dihydrochloride (a gift from K. Thomae, Federal Republic of Germany), and 4-DAMP (a gift from M. Kaliner, Bethesda, Md.).

Skin testing was performed by intradermal injection of 20 μ l of control and test reagents at various doses. Doses listed represent the total amount injected. The largest diameter of the wheal and flare, as well as the perpendicular diameter, were measured at 15 minutes, and all data are expressed as mean wheal and flare.

Statistical analysis

All data were analyzed with a database statistical analysis package for clinical research, CLINFO (Bolt, Beranek, and Newman, Inc., Cambridge, Mass.).

RESULTS

The mean wheal and flare responses of seven individuals tested with 20 nmol of both histamine and atropine are presented in Fig. 1. All subjects tested had a wheal-and-flare response to both histamine and atropine with mean histamine wheal/flare of 12.2/35.2 mm and mean atropine wheal/flare of 8.7/19.1 mm. Atropine-induced cutaneous responses varied by <10% in subjects skin tested multiple times on different days. Neither histamine nor atropine induced late cutaneous responses.

To determine if atropine-induced cutaneous responses were specific for atropine versus anticholinergic agents in general, we skin tested five subjects with both atropine and scopolamine (Fig. 2). All subjects also had an immediate wheal and flare to 20 nmol of scopolamine. However, cutaneous wheal-and-flare responses were not elicited with 2 nmol of scopolamine, whereas 2 nmol of atropine still induced a large wheal and flare. Skin testing with ipratropium bromide, a synthetic quaternary ammonium anticholinergic agent, yielded similar results to those found with scopolamine.

To ascertain if mast cells might in part be responsible for anticholinergic-induced cutaneous responses, we evaluated the effect of treatment with antihistamine on atropine-induced wheal and flare. Four subjects were skin tested with 20 nmol of histamine and atropine and subsequently were administered terfenadine, 60 mg, every 12 hours for three doses. Skin testing to histamine and atropine was then repeated with the results expressed as the percent inhibition of cutaneous response. This was calculated as (initial response - response after antihistamine)/(initial response) $\times$ 100%. Terfenadine sig-

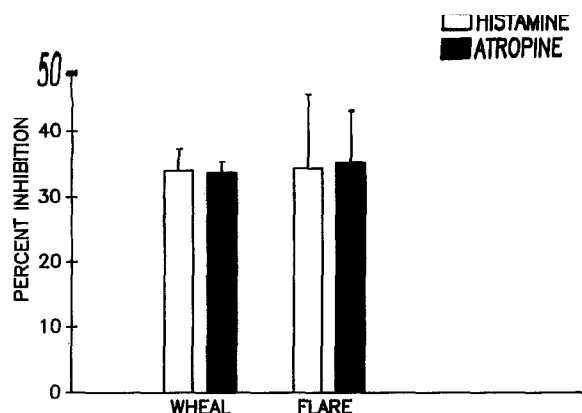


FIG. 3. Effect of antihistamine on atropine-induced cutaneous responses. Four subjects were skin tested with 20 nmol of histamine and atropine and then were administered terfenadine, 60 mg, every 12 hours for three doses. Skin testing was repeated, and the percent inhibition caused by antihistamine was calculated. Both histamine- and atropine-induced wheal-and-flare responses were significantly inhibited ($p < 0.05$ by Student's paired t test) by the antihistamine, terfenadine.

nificantly ($p < 0.05$) and equivalently inhibited both histamine- and atropine-induced cutaneous wheal-and-flare responses by 25% to 35% (Fig. 3). These data suggest a possible role for mast cell degranulation in anticholinergic-induced immediate skin test responses.

We next performed a series of skin tests to determine the potential role of muscarinic receptor subtypes in anticholinergic-induced cutaneous responses. The dose-response results of skin testing with the nonspecific muscarinic antagonist, atropine, the M1-specific antagonist, pirenzepine, the M2 specific antagonist, AFDX-116, and the M3-specific antagonist, 4-DAMP, are illustrated in Fig. 4. At all doses tested, atropine-induced cutaneous responses were larger than the responses induced by the three specific muscarinic receptor subtype antagonists. Cutaneous wheal responses induced by pirenzepine and 4-DAMP were relatively equivalent and larger than responses induced by AFDX-116 at doses < 200 nmol. At 200 nmol, all three muscarinic receptor subtype antagonists induced equivalent wheal responses.

It should be noted that the three muscarinic receptor subtype antagonists have variable affinities for their respective receptors and that they are not completely specific for one receptor subtype.⁵⁻⁷ Moreover, atropine has a relatively higher affinity for each of the three receptor subtypes. From radioligand binding data reported previously, atropine has a tenfold to 100-fold greater affinity for M1 receptors versus pirenzepine, a 100-fold greater affinity for M2 receptors versus AFDX-116, and an equivalent affinity for M3

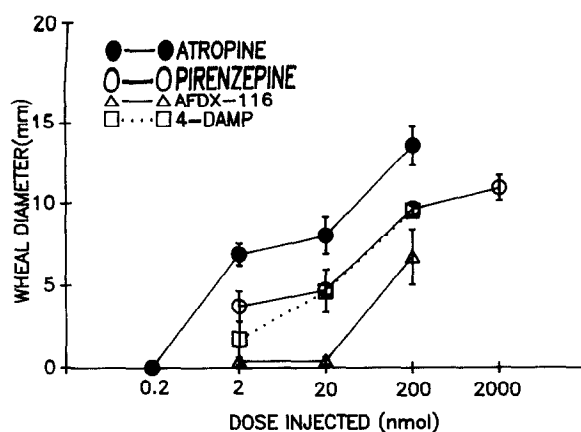


FIG. 4. Dose responses of specific muscarinic receptor antagonists. Agents listed were injected intradermally, and the mean wheal diameter was measured at 15 minutes. Results represent data from five subjects.

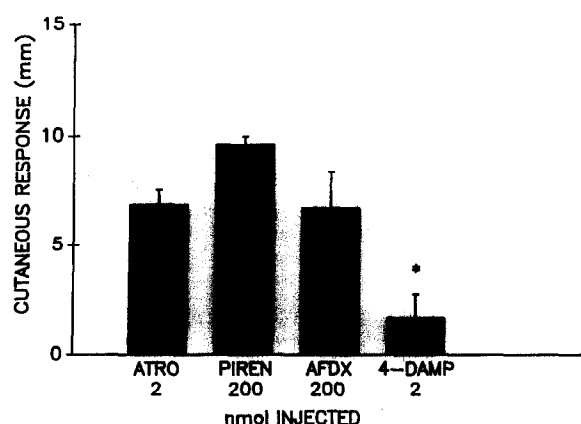


FIG. 5. Cutaneous responses to equivalent doses of muscarinic antagonists. Five subjects were skin tested with equivalent doses of atropine (ATRO), pirenzepine (PIREN), AFDX-116 (AFDX), and 4-DAMP based on their relative affinities for the specific receptor subtype in question (see text for details). Results represent mean wheal diameter measured at 15 minutes in five subjects; *significantly different from other agents with $p < 0.01$.

receptors versus 4-DAMP.⁵⁻⁷ Thus, to further evaluate whether a particular receptor subtype might be important in anticholinergic-induced cutaneous responses, we compared the cutaneous wheal responses produced by atropine to wheal responses produced by 100-fold more pirenzepine and AFDX-116, and an equivalent amount of 4-DAMP. We based these doses on the relative affinities for these agents to the specific muscarinic receptor subtypes.⁵⁻⁷ The results from skin testing of five healthy volunteers to 2 nmol of atropine, 200 nmol of pirenzepine, 200 nmol of AFDX-116, and 2 nmol of 4-DAMP are illustrated in Fig. 5. Equiv-

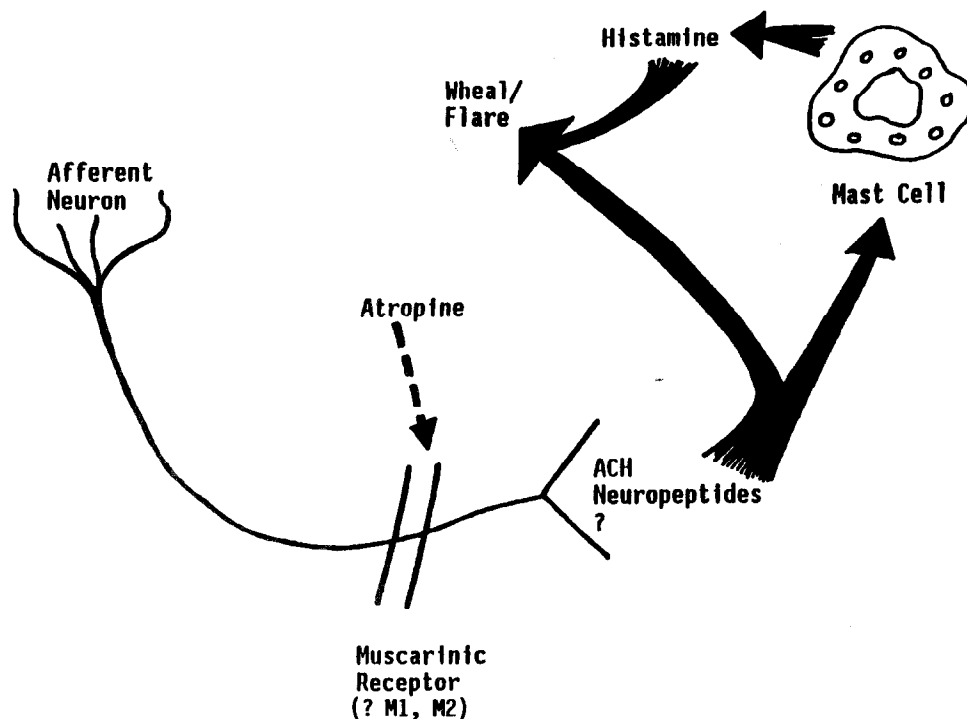


FIG. 6. Proposed mechanism of antimuscarinic-induced cutaneous responses. Atropine blocks specific inhibitory muscarinic autoreceptors leading to the release of acetylcholine, neuropeptides, and other neurotransmitters. These neurotransmitters then produce a wheal and flare by direct action and/or by indirectly causing mast cells to degranulate.

alent cutaneous wheal responses were induced by atropine, pirenzepine, and AFDX-116. 4-DAMP, however, produced significantly smaller cutaneous responses than the other three agents. This comparison suggests that an M1 or M2, but not an M3, muscarinic receptor subtype may be important in anticholinergic-induced cutaneous wheal responses.

DISCUSSION

Based on results of this study, we conclude that cutaneous wheal-and-flare responses to anticholinergic agents cannot effectively be used as an indicator of IgE-mediated hypersensitivity to these agents. All our subjects had a positive immediate skin test to atropine and the other anticholinergic agents tested. Cutaneous responses to atropine were significantly larger than responses induced by scopolamine or ipratropium bromide. The etiology of this difference is unknown but may be due to varying affinities for the specific muscarinic receptor subtypes.

To our knowledge, cholinergic/anticholinergic effects on isolated pure populations of cutaneous mast cells have not been previously evaluated. However, previous studies with rat peritoneal mast cells, which appear to be structurally and functionally similar to

cutaneous mast cells, have demonstrated histamine release in response to the cholinergic agonist acetylcholine.^{8,9} This response can be blocked by atropine, suggesting the presence of muscarinic receptors on these mast cells. Furthermore, cholinergic agonists produce a wheal-and-flare response when they are injected into the skin of humans. We do not believe that anticholinergic agents are acting as direct histamine releasers since it would be unlikely that both agonists and antagonists for a specific receptor would directly cause mast cell-mediator release. We were able, however, to demonstrate significant suppression of wheal-and-flare response to anticholinergics after treatment with antihistamine (Fig. 3), supporting a potential role of mast cell degranulation in this process.

To understand how both an agonist and an antagonist for a given receptor produce a wheal-and-flare response, we must first review the concept of muscarinic receptor subtypes. To date, there are three pharmacologically defined muscarinic receptor subtypes.^{5-7, 10-14} The M1 receptor has been best characterized in neural tissue and has been demonstrated to stimulate vagal neural transmission in airway tissues.^{5, 11} Specific high-affinity antagonists for this receptor subtype include pirenzepine, telenzepine, and

dicyclomine. The M2 receptor has been best characterized in cardiac tissue but is also found in neural tissue and smooth muscle throughout the body. In the airways, it is believed to function as an "autoreceptor," reducing vagal neurotransmission.^{11, 14} Specific antagonists for the M2 receptor subtype include gallamine and AFDX-116. The M3 receptor has been well characterized in exocrine tissue, smooth muscle, and neural tissues and is believed to mediate airway smooth muscle constriction.^{5, 11} Specific antagonists for this muscarinic receptor subtype include 4-DAMP and hexahydrostiladifenidol (HHSD).^{5-7, 10} Thus, in the airways, antagonists to muscarinic receptors might mediate both bronchodilation (M1 and M3 receptors) and bronchoconstriction (M2 receptors). We propose the existence of muscarinic receptor subtypes in the skin, similar to those proposed to exist in airways.^{11, 14} Muscarinic autoreceptors would inhibit neurotransmission and the subsequent release of acetylcholine. By blocking these receptors, atropine and the specific muscarinic receptor subtype antagonists that we have tested would increase neurotransmission, leading to the release of neurotransmitters, including acetylcholine. The released neurotransmitters would ultimately produce positive cutaneous responses.

The proposed inhibitory muscarinic autoreceptors in airway tissue are of the M2 subtype.^{11, 14} There is, however, known to be significant heterogeneity among the function of these specific receptor subtypes in various tissues throughout the body.^{5-7, 10, 12, 13} By using specific muscarinic receptor subtype antagonists, pirenzepine, AFDX-116, and 4-DAMP, we attempted to determine which muscarinic receptor subtypes are important in producing cutaneous responses to anticholinergic agents. At equimolar doses, atropine-induced cutaneous wheal responses were larger than responses elicited by the three specific muscarinic receptor subtypes. This finding is not surprising since the affinity of atropine for each of the three muscarinic receptor subtypes is greater than the affinity of the specific antagonists for their respective receptors. Although they represent the most selective muscarinic subtype antagonists currently available, pirenzepine, AFDX-116, and 4-DAMP bind with variable affinity to each subtype of muscarinic receptor.^{5-7, 10, 12, 13} We attempted to compensate for this variable receptor affinity by performing the study outlined in Fig. 5. Specifically, we used doses of the antagonists for each of the three receptor subtypes that should have approximated an equivalent dose of atropine for the three receptor subtypes. Based on previously reported radioligand binding data with rat hippocampus (M1), atria (M2), and submandibular glands (M3), 2 nmol of atropine should have an equivalent effect as 200

nmol of pirenzepine, 200 nmol of AFDX-116, and 2 nmol of 4-DAMP on M1, M2, and M3 receptors, respectively.⁵⁻⁷ At these doses, the cutaneous wheal responses to atropine, pirenzepine, and AFDX-116 were relatively equivalent and significantly larger than responses induced by 4-DAMP. It should be noted, however, that the affinity of a ligand for a receptor may not predict its ultimate physiologic potency to induce a receptor-mediated event. Moreover, intradermal skin testing may not be sensitive enough to definitively delineate which specific subtype of muscarinic receptor mediates the cutaneous responses. Nonetheless, these data suggest that M1 and M2, but not M3, receptor subtypes may be involved in anticholinergic-induced cutaneous responses.

Based on the information from this and other studies, we propose the following mechanism for anticholinergic-induced cutaneous responses (Fig. 6). Atropine blocks an afferent nerve autoreceptor of muscarinic receptor M1 or M2 subtype, leading to the release of acetylcholine, neuropeptides, and possibly other neurotransmitters. The neurotransmitters could then either cause mast cell degranulation and/or directly act on cutaneous blood vessels leading to a wheal and flare. Since antihistamines partially abrogated cutaneous responses, it is likely that mast cells are involved. In this regard, both acetylcholine and neuropeptides can cause cutaneous mast cells to degranulate.^{8, 9, 15} It is hoped that with the development of more specific antagonists for muscarinic receptor subtypes the exact mechanisms involved in anticholinergic-induced cutaneous responses can be delineated in the future. Nonetheless, these data indicate that anticholinergics cause a positive wheal-and-flare response and thus can not be used to screen for allergic reactions to these drugs.

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An open-label study of high-dose intravenous immunoglobulin in severe childhood asthma

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Eight pediatric patients with severe steroid-dependent asthma were enrolled in an open-label trial of high-dose intravenous immunoglobulin (IVIG) in an attempt to decrease their steroid requirements. Monthly therapy with high-dose IVIG resulted in a threefold decrease in both maintenance oral corticosteroid dose and in extra oral corticosteroids needed for control of exacerbations of asthma. This was accompanied by significant improvements in peak expiratory flow rates and in symptom-score rating. An immunomodulatory effect of IVIG was suggested by the changes in immediate skin test reactivity. Seven of the eight patients demonstrated one or more reactions to a panel of allergens before therapy. During the course of the trial, there was a progressive diminution in skin test reactivity with a 100-fold reduction in sensitivity at the completion of 6 months of therapy. In this preliminary study, the reduction in steroid requirements, improvement in symptoms and peak flow measurements, and diminution in immediate skin test reactivity support a potential role for IVIG in the treatment of severe steroid-dependent asthma. A larger, randomized trial now appears warranted. (J ALLERGY CLIN IMMUNOL 1991;87:976-83.)

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Abbreviations used

IVIG:	Intravenous immunoglobulin
PEFR:	Peak expiratory flow rate
ANOVA:	Analysis of variance
LD:	Low dose
HD:	High dose
IT:	Immunotherapy
PC ₂₀ :	Provocative concentration causing a 20% fall in FEV ₁
Ag:	Antigen
Ab:	Antibody
PLSD:	Protected least-significant difference
CI:	Confidence interval
SPT:	Skin prick test