Response of nasal mucosa to histamine or methacholine challenge: Use of a quantitative method to examine the modulatory effects of atropine and ipratropium bromide

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We have developed a new technique for the direct local administration of test solutions to the nasal mucosa and for quantification of nasal secretory responses. This technique, a variation on several published reports of filter paper use, allows simple and rapid determination of drug effects and facilitates the analysis of ipsilateral and contralateral responses to local challenge of the nasal mucosa. We have used this technique to investigate the secretory responses of the nasal mucosa to methacholine and histamine and to determine the effects of atropine and ipratropium bromide (Atrovent nasal spray) on these secretory responses. (J ALLERGY CLIN IMMUNOL 1992;90:1051-4.)

Key words: *Nasal mucosa, filter paper disks, methacholine, histamine, ipratropium bromide*

Various techniques have been used to measure the effects of pharmacologic agents on the nasal mucosa. The monitoring of mucosal responses has been accomplished with the use of methods such as measuring changes in airway resistance,[1-3] collecting and quantifying secretions with a funnel or similar device,[4-6] counting tissues,[7] and assessing patients' symptoms.

The use of filter paper strips or disks as carriers for nasal application of drugs or antigens or as a means of collecting nasal secretions has been reported by several investigators.[5, 8-13] We have combined and adapted these filter paper techniques to examine the effects of local stimulation of the nasal mucosa and to reproducibly quantitate the response to such stimulation. Our methodology with filter paper disks for these purposes has several advantages over other methods.

TECHNIQUE

Filter paper disks are punched out of filter cards by an 8 mm hole punch. Disks are prepared from white filter stock of 1.2 mm thickness and from brown 0.9 mm thick stock. White disks, which are used for mucosal stimulation, can hold 50 μl of solution, whereas brown disks have a 35 μl capacity. Brown disks are placed in 1.5 ml plastic microtubes and preweighed. White disks are saturated with challenge solution or with pH- and osmolarity-matched control solutions.

During careful inspection of the nasal cavity with a nasal speculum and a surgical headlight, crusts are removed with Duckbill forceps, and secretions are dried by applying a white disk. Challenge disks are then placed on either the middle third of the anterior portion of the nasal septum, just posterior to the mucocutaneous junction, or on the medial surface of the anterior portion of the inferior turbinate.

White disks saturated with 50 μl of challenge or control solution are left in place for 1 minute. After 30 seconds a preweighed brown disk is applied to the same area for 30 seconds to collect secretions. The brown disks are then returned to their preweighed microtubes, which are capped and reweighed. The difference in weights reflects the amount of secretions collected in a fixed period of time.

This technique has several advantages over other methods. The procedure is relatively simple and highly quantitative. Measurements can be obtained rapidly and at intervals as frequent as once per minute to monitor the time course of a response. Another advantage is the ability to compare ipsilateral responses to those occurring on the contralateral side of the nasal cavity.

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PERENNIAL RHINITIS

The submucous glands are major contributors to nasal secretions. These glands receive abundant innervation from the parasympathetic nervous system.[14] The notion that the hypersecretory response of patients with perennial rhinitis is a result of increased parasympathetic activity is supported by the actions of methacholine. Experimental evidence indicates that methacholine, a parasympathomimetic agent, induces greater reactivity of the nasal mucosa in subjects with perennial rhinitis when compared with normal control subjects.[4, 15] Anticholinergic drugs block the parasympathetic induction of secretion and show promise for the treatment of excessive rhinorrhea in patients with perennial rhinitis.

To further our understanding of the pathophysiology of perennial rhinitis and assess the potential of anticholinergic therapy, we have used our filter disk technique in placebo-controlled, double-blind experiments to study the effects of methacholine, histamine, atropine, and ipratropium bromide on nasal secretion.

EFFECTS OF ATROPINE AND IPRATROPIUM BROMIDE ON THE NASAL RESPONSE TO METHACHOLINE AND HISTAMINE

Effects of methacholine

Methacholine is a synthetic analog of acetylcholine. The filter disk method was used to assess the effects of methacholine in terms of kinetics, dose-response relationship, ipsilateral versus contralateral responses, and the development of tachyphylaxis.[16]

The ipsilateral secretory response to methacholine challenge reaches maximal intensity within 30 seconds and decreases to baseline levels within 3 to 5 minutes. This response is seen when doses of 1.7 or 0.6 mg per filter disk (in 50 μl of saline solution) are used. A sham challenge of saline solution alone preceded the methacholine challenge by 5 minutes in these experiments and had no apparent effect on secretion magnitude when compared with the response after methacholine.

There was a dose-dependent increase in nasal secretion weight from 5.9 ± 0.9 mg after sham challenge with saline solution to a maximum of 24.9 ± 2.2 mg after the highest 5 mg dose of methacholine. This overall increase in secretion weight was significant ($p = 0.0001$). The weights of secretions after all five doses used (0.19, 0.6, 1.7, and 5 mg of methacholine) were significantly increased over that of saline solution ($p \leq 0.05$).

The challenge technique was shown to be reproducible. Three dose-response curves, which were separated by at least 2 days, were performed on each of nine patients. The coefficients of variation of these values ranged from 3.3% to 22.2%, with a mean of 12.1%.

No significant increases in secretions on the contralateral side were seen in response to ipsilateral stimulation with methacholine. An overall significant difference between the curves of the ipsilateral and contralateral responses was found ($p = 0.00001$).

Repeated stimulation of the nasal septum five times with 1.7 mg of methacholine showed no significant difference between the response after the first dose compared with the last four. This result indicated a lack of tachyphylaxis in response to the repeated administration of methacholine.

Effects of histamine

The filter disk method was also used to measure the effects of the natural autacoid, histamine, on the nasal mucosa. Several differences were noted between the responses to methacholine and histamine. The most notable differences were in the contralateral responses and the development of tachyphylaxis.

The kinetics of the secretory response to histamine were very similar to that seen with methacholine. Doses of 0.04 or 1 mg induced maximal response within 30 seconds, and secretions returned to control levels within 5 minutes. As in the experiments used to determine the kinetics of the response to methacholine, sham challenge with saline solution alone preceded histamine challenge by 5 minutes and had no apparent effect on secretion magnitude when compared with the response after histamine.

As was seen with methacholine, a dose-dependent increase in nasal secretion weight was seen for histamine. Doses of 0.004, 0.01, 0.04, 0.1, 0.3, 1, and 3 mg of histamine were used. Nasal secretion weights increased from 4.6 ± 0.6 mg after saline solution to a maximum of 26.8 ± 2.3 mg after the highest dose of histamine. The overall increase in weights was also significant ($p = 0.0001$), and all secretion weights after histamine doses of 0.04 mg or more were significantly increased over the baseline saline challenge ($p \leq 0.05$).

The reproducibility of the response to histamine challenge was tested by performing three histamine dose-response curves separated by at least 1 week on each of eight patients. The coefficients of variation had a mean of 20.6%.

In contrast to the lack of reflex response seen after methacholine challenge, stimulation with doses of 0.3 mg or more of histamine induced a significant ($p \leq 0.05$) increase in the weight of contralateral secretions. Secretion weights increased from 3.9 ± 0.9

mg after saline solution to 11.8 ± 1.8 after the maximal dose of histamine, but this contralateral response was significantly lower in magnitude than the ipsilateral response ($p = 0.00001$).

After repeated challenges with 1 mg of histamine, responses after the last three doses were significantly lower ($p \leq 0.05$) than those after the first two doses, which demonstrates tachyphylaxis after the fifth repeated dose. Tachyphylaxis occurred earlier on the contralateral side after repeated histamine challenge and was evident after the third repeated dose.

Effects of premedication with atropine

The septal mucosa was challenged with methacholine without and after topical premedication with 0.3 mg of atropine. A significant decrease occurred in methacholine-induced secretions after premedication with atropine at all doses, including saline challenge ($p \leq 0.05$).

Despite premedication with atropine, a significant (although reduced) increase occurred in secretions ($p \leq 0.05$ compared with saline) after the two highest doses of methacholine (1.7 and 5 mg), which suggests that high levels of parasympathetic activity might overcome the inhibitory effect of atropine.

Because histamine induced both contralateral and ipsilateral increases in nasal secretion, saline solution, as placebo or containing atropine, was sprayed on either one or both sides of the nasal cavity. After increasing doses of histamine, secretions were collected from both sides. After bilateral application of atropine, secretions were significantly reduced on both sides (ipsilateral $p = 0.003$; contralateral $p = 0.01$). After premedication with atropine, the ipsilateral response was reduced by 44%, and the contralateral response was reduced by 77% compared with placebo.

Premedication of the nasal mucosa with atropine only on the side of histamine challenge produced partial inhibition of ipsilateral secretions but no inhibition of contralateral secretions.

Effects of premedication with ipratropium bromide

The systemic effects of atropine limit its therapeutic use for the treatment of rhinorrhea in most cases. Ipratropium bromide is an anticholinergic agent that is not absorbed systemically and yields local therapeutic effects without systemic effects.

Dose response. Premedication of the nose with 21, 42, 84, or 168 μg of ipratropium bromide 1 hour before methacholine challenge led to significant reductions in the levels of nasal secretions at all doses compared with placebo ($p = 0.01$).[17] Results were similar to those seen with atropine. The highest doses of methacholine increased nasal secretions, although to levels significantly below those seen after placebo pretreatment. The effects of ipratropium pretreatment were dose dependent. The therapeutic effect increased with increasing levels of ipratropium and showed no signs of leveling off, even at the highest dose used (168 μg), which was significantly more effective than the three lower doses of the drug ($p = 0.01$).

DISCUSSION

A new technique

We have developed a new technique for localized application of test solutions to the nasal mucosa and quantitation of the secretory response to such mucosal challenge. This filter disk technique has several advantages over previous methods of studying the response of the nasal mucosa to challenge. The use of filter disks allows precise localization of the challenge solution. Rapid, quantitative determination of local nasal secretions is simplified, and kinetic analysis is facilitated by the ease and rapidity with which serial determinations can be carried out with this method. In addition, the highly localized nature of the filter disk methodology enables comparison of ipsilateral versus contralateral responses.

New insights regarding the different activities of histamine and methacholine

The use of our filter disk technique has allowed us to obtain new insights into the effects of histamine and methacholine. Histamine but not methacholine induces reflex secretory activity on the contralateral side of the nasal cavity. Atropine inhibits this contralateral reflex response. The response to histamine but not methacholine undergoes tachyphylaxis. Finally, ipratropium bromide and atropine reduce the secretory response to methacholine.

The inhibitory effect of atropine on contralateral secretion after histamine challenge suggests that this reflex is mediated parasympathetically.

The parasympathetic agonist activity of methacoline that induces secretion does not undergo tachyphylaxis. Thus the response to histamine, while apparently caused in part by an atropine inhibitable parasympathetic component, is clearly more complex than the response to methacholine. The tachyphylaxis seen after repeated histamine challenge is likely caused by the neural component of the response to histamine.

CONCLUSION

The anatomic division of the nose into two parts and the ease of access to the anterior nasal cavity

suggested a novel use of filter paper disks for the administration of test solutions and quantification of secretory responses. Using this new technique, we have been able to confirm the effectiveness of the anticholinergic agents atropine and ipratropium bromide for inhibiting the secretory response to the parasympathetic agonist methacholine and the autacoid agent histamine. We have also demonstrated that nasal challenge with methacholine or histamine induces a dose-dependent increase in nasal secretion. Finally, we have shown that histamine but not methacholine induces parasympathetically mediated reflex secretory activity on the contralateral side of the nasal cavity and that the secretory response to histamine but not to methacholine undergoes tachyphylaxis.

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