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Demonstration of bilateral cholinergic secretory response after unilateral nasal cold, dry air challenge* t

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SUMMARY

Cold, dry air (CDA) causes rhinorrhoea and nasal congestion in some individuals. This response can be mimicked in the laboratory by exposing susceptible individuals to cold, dry air nasal breathing. One of the characteristics of this response is that nasal secretions are produced by both nostrils after a unilateral challenge. This study evaluated the role of cholinergic innervation on the ipsi- and contralateral responses to unilateral CDA challenge. Twelve individuals participated in a randomized, double-blind, placebo-controlled, 3-way crossover study where local atropine and placebo were alternated ipsilateral and the contralateral to the CDA challenge. The reproducibility of the model, assessed by the response after pre-treating with placebo, was excellent; after placebo, the ipsilateral response was double the size of the contralateral. Regardless of the site of application, atropine significantly reduced the secretory response to CDA by 60-70%. However, significant secretions were still induced by CDA, even after atropine treatment. We conclude that cholinergically-mediated neuronal pathways play a major role in the nasal secretory response to CDA. Additional neuronal pathways may, however, be involved. This method is a tool to understand the different components of the mucosal response to a cold and dry environment.

Key words: dry air challenge, nasal secretion, cholinergic innervation,

INTRODUCTION

Nasal challenges with cold, dry air (CDA) can reproduce the symptoms of patients with prior history of rhinorrhoea and congestion upon natural exposure to cold and windy environment. In this experimental model, the levels of histamine, prostaglandin D2 and mast-cell tryptase increase in nasal secretions during the acute response, suggesting mast cell activation (Togias et al., 1985; Proud et al., unpublished data). This non-antigenic mast cell stimulus can also lead to a late-phase reaction exhibiting patterns similar to those of the late phase after allergen challenge (Iliopoulos et al., 1988). However, despite the similarities in clinical and biochemical parameters, the pharmacological modulation of the nasal reaction to **CDA** is somewhat different from that of the reaction to allergen. In previous attempts, we found no effect of a topical antihistamine, azatadine base (Togias et al., 1987), nor of topical steroids, beclomethasone (Cruz et al., 1991), on the acute nasal response to CDA. However, a significant inhibitory effect of atropine was observed (Cruz et al., 1990). This finding was in agreement with clinical studies demonstrating efficacy of ipratropium bromide, an acetylcholine antagonist, in cold-air-induced rhinorrhoea (Ostberg et al., 1987; Silvers et al., 1988). These studies suggest that cholinergic neuronal activity contributes to the nasal CDA reaction.

The relative role of mast cell mediators in the nasal response to CDA is not clear. We have strong evidence to suggest that the activation of mast cells results from a direct effect of the CDA-induced hypertonic environment in the nasal mucosa (Togias et al., 1988). It is unknown, however,

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whether the atropine-sensitive component of the reaction to CDA derives from central or local neuronal activity. Recently, we have reported that unilateral nasal provocation with CDA results in bilateral secretory activity suggesting a central neurogenic reflex (Philip et al., 1991). This study was designed to evaluate the role of cholinergic activity on the contralateral reflex response to unilateral CDA challenge. Our results confirm that unilateral CDA challenge induces a bilateral secretory response with the ipsilateral response being greater than the contralateral. We also show that atropine is a potent inhibitor of both the ipsi- and the contralateral responses.

METHODS

Study design

Twelve volunteers (11 males and 1 female) between the ages of 27 and 38 years were involved in the study. All volunteers reported a history of rhinorrhoea and congestion upon exposure to cold and windy environments. All also had a prior positive nasal reaction to CDA challenge, defined by the development of rhinorrhoea and at least a two-fold increase over baseline in the levels of histamine and/or TAME-esterase activity in recovered nasal fluids. Seven of the subjects were allergic, but asymptomatic at the time of the study; no subject was on medication. All subjects gave informed consent before entry, and the study was approved by the Institutional Review Board of the Francis Scott Key Medical Center.

The study was a double-blind, randomized, placebo-controlled, 3-way crossover design. Nasal provocation with CDA was performed unilaterally 10 min after topical pretreatment of the nose according to one of the following protocols: (A) placebo on both the ipsi- and the contralateral sides; (B) atropine ipsilateral and placebo contralateral to the challenge side; and (C) atropine contralateral and placebo ipsilateral to the challenge side. Each challenge was separated by a wash-out period of at least 72 h. Atropine sulfate (Elkins-Sinn Inc., Cherry Hill, NJ, USA) at a concentration of 0.5 mg/ml, and placebo (normal saline solution used as diluent for the preparation of atropine solutions), were delivered by a metered-pump spray in each nostril. Three doses of 0.145 ml each were sprayed every 5 min to deliver a total dose of 0.25 mg atropine. This dose had been found to be effective in a preliminary dose-response study.

Nasal challenge

To establish stable baseline conditions, five preliminary nasal lavages with 5 ml normal saline per nostril at 37 °C were performed; the returned fluid was discarded (Figure 1). Filter paper discs placed bilaterally on the anterior part of the nasal septum were used to collect secretions on both sides. To remove any remaining saline, the first collection disc was discarded. Baseline secretions (discs pre-drug) were obtained 3 min later. After delivering the study medication, another disc was used to blot away secretions; 3 min later, a collection disc was placed for post drug baseline measurements (discs post-drug). Then, the left nostril was sealed with a silicone putty plug and a 12-min unilateral cold, dry air challenge was delivered to the right nostril through a nasal CPAP mask tightly covering the nose. Patients were asked to inhale through the nose, and to exhale through the mouth. Compressed air from a tank was delivered at a flow of 26 l/min, at a temperature ranging from 0-5 °C, and at a relative humidity of less than 10%. Post-CDA secretions were measured bilaterally with additionnal collection discs, 1, 5, and 9 min after the end of the challenge (discs post-CDA 1, 5, and 9).

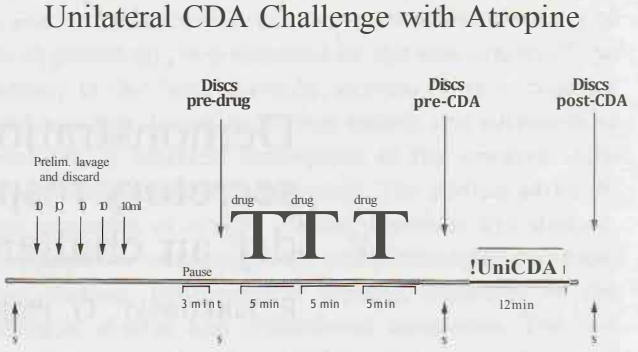

Figure 1. Protocol: Unilateral cold, dry air nasal challenge after double-blind, placebo-controlled pre-treatment with topical atropine.

Measurements of secretions

Filter paper discs for the collection of secretions were punched out from Shandon filter cards using an 8-mm hole puncher, preweighed and stored in 1.5 ml microtubes (Sarstedt, Inc. Newton, NC, USA). Secretions were always collected from the same area of the septal mucosa. The discs were placed on the mucosa under visual control, using a surgical headlight, a speculum and a duckbill forceps. Discs were applied for 30 s, removed, replaced in the microtube and reweighed. The weight of secretions was calculated by subtracting the pre- from the post-collection weights.

Statistical analysis

Data are presented as mean± standard error of the mean (SEM). Nonparametric statistics were applied. Friedman two-way analysis of variance was performed on the net increase of the secretory response (over baseline) for the ipsilateral or contralateral side in all 3 protocols. Post-hoc analysis employed the Wilcoxon's matched-pairs signed-rank test.

RESULTS

Atropine sprayed into the nasal mucosa may be systemically absorbed and could interfere with the secretory response of the untreated side. In a preliminary dose-response study using the protocol depicted in Figure 1 with 5 of the 12 CDA-responders, we found that pretreating the challenge side with 0.25 mg of atropine inhibited the ipsilateral secre-

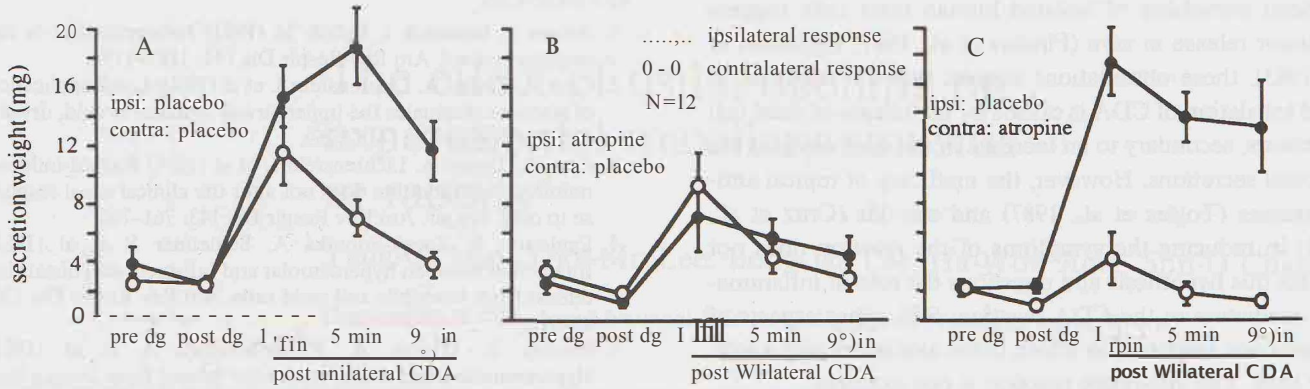

Figure 2. Bilateral secretory response after unilateral cold, dry air (CDA) nasal challenge. A: Both sides treated with placebo; B: Atropine ipsilateral and placebo contralateral to the challenge; C: Placebo ipsilateral and atropine contralateral to the challenge.

tory response without affecting the contralateral response. With a 0.5-mg dose, there was evidence of a contralateral inhibitory effect. We, therefore, used the 0.25-mg dose for the main study.

Our results indicate that both the ipsi- and contralateral secretory responses after unilateral CDA challenge are, to a significant degree, cholinergically mediated (Figure 2).

With placebo on both sides, unilateral CDA challenge showed a significant increase in the secretion weights on both the ipsi- and contralateral sides (Figure 2A). There was no significant difference between ipsi- and contralateral secretion weights 1 min after the end of the challenge ($p=0.15$), but the contralateral response decreased more rapidly (at 1 and 5 min ipsi- vs contralateral: $p<0.009$), almost reaching baseline after 9 min. The total amount of secretion collected at 1, 5, and 9 min on the contralateral side represented 50% of the challenged side.

Pretreatment of the challenged side with atropine led to a significant decrease in secretion weights (Figure 2B). Baseline secretion (pre-dg) was significantly reduced after atropine (post-dg; $p=0.01$). Compared to placebo (Figure 2A), atropine reduced the CDA-induced ipsilateral secretory response at 1 min ($p=0.04$), as well as the total response calculated by the addition of the values obtained at 1, 5, and 9 min ($p=0.01$). The total amount of secretion from the 1-, 5-, and 9-min time points was reduced by 63% after atropine. However, even with atropine, secretion weights at 1, 5 and 9 min after CDA remained significantly higher than the baseline, post-atropine value ($p<0.003$). The contralateral placebo-pretreated side was not affected by the ipsilateral treatment with atropine.

Pretreatment of the non-challenged side with atropine also led to a significant decrease in secretion weights (Figure 2C). Baseline secretion (pre-dg) was significantly reduced after atropine (post-dg; $p=0.004$). Compared to placebo (Figure 2A), contralateral atropine reduced the net CDA-induced contralateral secretion at 1 min ($p=0.03$) as well as the sum of the net secretions from 1, 5, and 9 min ($p=0.02$). Atropine reduced the total net secretions of the non-challenged side by 68%. Again, the 1-min post-CDA weight remained significantly different from baseline ($p=0.002$), even after atropine treatment. No difference in the response of the challenged, placebo-pretreated side between protocols A and C was found.

DISCUSSION

Unilateral CDA challenge induces a bilateral secretory response. The contralateral response is about 50% of the ipsilateral. Both ipsi- and contralateral secretory responses are, to a high degree, cholinergically mediated. Similar data, with respect to the effect of atropine, have been generated after histamine and antigen nasal challenge (Baroody and Naclerio, unpublished data).

The technique of unilateral CDA challenge may raise concerns regarding the unilaterality of the stimulus. Actually, the free posterior communication of the two nasal cavities could stimulate the plugged side. However, if this contralateral stimulus exists, its level must be far below the challenging stimulus, and the large amount of contralateral secretion is probably not generated solely through it. Using a nasal probe which carries a thermistor at the nasopharynx, we have preliminary data showing that the airstream temperature at the nasopharynx of CDA-responders during CDA challenge is above 25 °C. This suggests that the warming capacity of the challenged nostril is large enough to prevent the stimulation of the contralateral nostril from posteriorly leaking cold air.

Our results suggest that 60-70% of the CDA-induced secretions are generated through reflex mechanisms. Atropine, at the dose of 0.25 mg, inhibits 63% and 68% of the secretory response in the challenged and non-challenged side, respectively. One wonders whether a higher dose could produce more inhibition. Unfortunately, our preliminary study suggested that a 0.5-mg dose ipsilateral to the challenge could potentially affect the contralateral response, possibly via systemic absorption.

The mechani :m by which CDA challenge triggers the secretory response is not known. In previous experiments (Togias et al., 1988), we found that CDA-responders have significant increments in nasal fluid osmolality, which correlate with the release of inflammatory mediators such as histamine and TAME-esterase activity. Since increased

medium osmolality of isolated human mast cells triggers mediator release *in vitro* (Findlay et al., 1981; Eggleston et al., 1983), these observations suggest that the response to nasal inhalation of CDA is caused by the release of mast cell mediators, secondary to an increase in the osmolality of the mucosal secretions. However, the inefficacy of topical antihistamines (Togias et al., 1987) and steroids (Cruz et al., 1991) in reducing the symptoms of the reaction does not sustain this hypothesis and questions the role of inflammatory mediators in the CDA reaction. Still, other aspects of the mucosal response, in which these mediators play a role, may exist. The late-phase reaction is one example.

Since this, as well as our previous studies, indicates that nerves are activated, it would be interesting to examine what triggers the neuronal component. A possible explanation is that hyperosmolality or cooling affects sensory nerve endings. However, studies to examine this hypothesis have not been performed.

On the other hand, it appears that the effector neuronal pathway is not only cholinergic since a high dose of atropine failed to totally inhibit not only the ipsilateral, but also the contralateral secretory response. Although one can argue that a higher atropine dose would have been more effective, alternative neuronal pathways may exist. Neuropeptides are prime candidates. For example, Vasoactive Intestinal Polypeptide **(VIP)** is co-localized with acetylcholine in efferent nerve endings and may be released concurrently with the cholinergic mediator. This peptide may be responsible for the atropine-resistant contralateral secretory response. lpsilateral to the challenge, additional neuronal pathways may be involved. Activation of sensory nerves may not only lead to central but also to axon reflexes which can antidromically stimulate other sensory arborizations to release pro-inflammatory neuropeptides such as Substance P and Calcitonin-Gene-Related Peptide **(CGRP).** Both substances are present in human nasal mucosa (Barnes et al., 1991) and both have potent vasodilatatory properties. Substance P also activates human submucosal glands (Barnes et al., 1991). Unfortunately, as a result of the potent activity of degrading enzymes, the task of measuring neuropetides in nasal secretions after CDA provocation may be difficult.

In summary, this study clearly demonstrates that cholinergic signals are of major importance in the nasal secretory response to CDA. The significant advance is that we have developed the methodology to separate the purely neurogenic response, that of the contralateral to the challenge side. Our method is highly reproducible, as demonstrated in Figure 2, and can now be used to examine the effects of several other pharmacological agents on the nasal response to cold, dry air. This sets the stage for further information on the pathophysiology of this reaction.

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