

Effect of Topically Applied Atropine, Methysergide and Chlorpheniramine on Nasal Challenge with Serotonin

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Serotonin challenge in the nose produces sneezing and rhinorrhoea. To study the mode of action of serotonin in the nose, 14 normal persons, in a double-blind, randomized study, were provoked with serotonin in three concentrations. Fifteen minutes before challenge, they were pretreated in the nose with either a cholinocceptor antagonist (atropine), a serotonin antagonist (methysergide), an H_1 -histamine antagonist (chlorpheniramine) or isotonic saline. The number of sneezes was noted, and the amount of secretions measured. Serotonin induced dose-related increase in sneezing and hypersecretion ($P < 0.02$). Atropine pretreatment reduced the amount of secretion ($P < 0.05$ and $P < 0.1$), but had no effect on sneezing. Methysergide and chlorpheniramine had no effect on the serotonin-induced nasal symptoms. Unilateral challenge with serotonin induced secretions from both sides, suggesting that serotonin-induced nasal symptoms are partly reflex-mediated.

Key words: atropine; chlorpheniramine; methysergide; nasal challenge; rhinitis; serotonin

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We have recently reported that nasal challenge with serotonin induces a nasal response in normal individuals, i.e. itching, sneezes and secretion, but no change in nasal airway resistance (17). We have also found that histamine, methacholine and allergen provocation produce nasal secretions which contain serotonin in variable concentrations (16). Serotonin might therefore play a pathophysiological role in allergic rhinitis.

This report presents the results of the effect of a cholinocceptor antagonist (atropine), a serotonin antagonist (methysergide) and an H_1 -histamine antagonist (chlorpheniramine) on serotonin-induced nasal symptoms. It was studied whether serotonin induced nasal symptoms via a reflex mechanism by examining the secretory response to unilateral provocation.

MATERIAL AND METHODS

Subjects

Fourteen normal females participated in the study. The mean age was 34.3 years (range, 18-60 years). No one had a history of atopic disease, perennial rhinitis or chronic symptoms from the airways, neither had they had a common cold during the last 2 weeks. They did not use any medicine during the trial period.

Provoking agents

Serotonin creatinine sulphate (serotonin) (Sigma Chemicals), 0.8 mg/ml (S_1), 4 mg/ml (S_2) and 20 mg/ml (S_3) in a phosphate buffer was

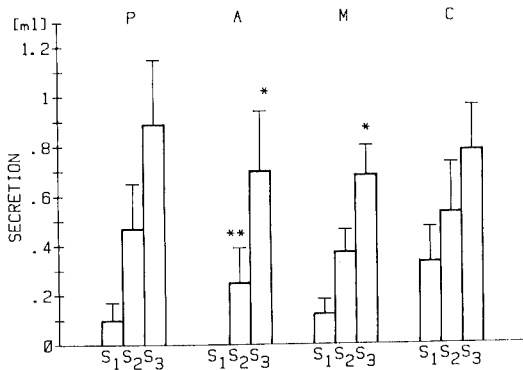


Fig. 1. The effect of different pretreatments on serotonin-induced nasal secretions in 14 normal subjects (treatment C only 9 persons) (mean + SEM). S₁ = serotonin, 0.8 mg/ml; S₂ = serotonin, 4 mg/ml; S₃ = serotonin, 20 mg/ml. P = isotonic saline; A = atropine; M = methysergide; C = chlorpheniramine. * $P < 0.1$; ** $P < 0.05$.

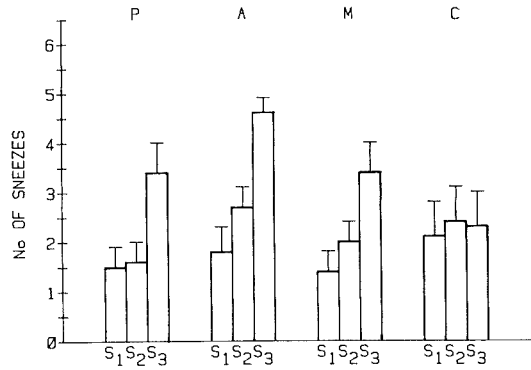


Fig. 2. The effect of different pretreatments on serotonin-induced sneezes in 14 normal persons (treatment C only 9 persons) (mean + SEM). S₁ = serotonin, 0.8 mg/ml; S₂ = serotonin, 4 mg/ml; S₃ = serotonin, 20 mg/ml. P = isotonic saline; A = atropine; M = methysergide; C = chlorpheniramine.

used; pH was measured to 7.3, 7.1 and 5.9, respectively. The dilutions were made the day before each trial day, and no preservatives were used.

For the nasal challenge a DeVilbiss No. 15 atomizer was used, which delivered 0.1 ml per actuation.

As pretreatment was used: atropine sulphate, 0.5 mg/ml (pH 6.7), methysergide maleate, 0.5 mg/ml (pH 5.1) and chlorpheniramine maleate, 3 mg/ml (pH 6.4). All drugs were in a phosphate buffer. Isotonic saline was used as placebo. The pretreatment solutions were coded, and the trial was undertaken with a double-blind design.

Nasal challenge

The provocation tests were carried out at the same time of day. The pretreatment drugs were used in a randomized order. Each trial day started with either atropine (0.2 mg), methysergide (0.2 mg), chlorpheniramine (1.2 mg) or isotonic saline, two puffs into each nostril. The subjects blew their noses after 15 min. At first, a control provocation was done with phosphate buffer 0.1 ml bilaterally. Increasing doses of serotonin (0.16 mg, 0.8 mg and 4 mg) were then given with 5-min intervals, one puff into each nostril. In each 5-min period, just after the

provocation, the subject bent her head forward, and after 5 min blew her nose into a funnel to collect the secretion, which was directly measured in ml.

In 10 persons 2 puffs of serotonin, 20 mg/ml, were given into the right nostril, and the amount of secretion from each nostril was collected over the next 5 min into two separate funnels.

Statistical methods

Friedman's test and the Wilcoxon's sign rank test for paired data were used.

RESULTS

No secretion was obtained in the 5-min period following the phosphate buffer control challenge. After pretreatment with placebo there was a dose-dependent increase in response to serotonin with regard to sneezes and secretions ($P < 0.02$) (Figs. 1, 2). Sneezes occurred promptly after most challenges, and hypersecretion followed. A few patients experienced slight and transient nasal blockage. The mean of the sum of secretions (S₁ + S₂ + S₃) after the different pretreatments was: 1.5 ml (placebo), 1.0 ml (atropine), 1.2 ml (methysergide) and 1.7 ml (chlorpheniramine). There was a statisti-

cal difference between total secretions ($P < 0.0005$, Friedman's test, $\chi^2 = 131$). Compared with placebo pretreatment, only atropine reduced the secretions after serotonin challenge (S_2 ; $P < 0.05$, S_3 ; $P < 0.1$, $S_1 + S_2 + S_3$; $P < 0.05$, Wilcoxon's test). Methysergide and chlorpheniramine did not influence the nasal response to serotonin.

In nine of the 14 persons, nasal challenge with serotonin in the high dose was repeated with 1 week's interval. The coefficient of variation was 0.04 for secretion and 0.31 for sneezes.

The application of serotonin unilaterally produced $0.24 \text{ ml} \pm 0.18$ (mean $\pm$ SD) of secretions on the same side, versus $0.06 \text{ ml} \pm 0.09$ (mean $\pm$ SD) on the other side.

DISCUSSION

As recently reported by us (17), nasal provocation by serotonin induces itching, sneezes and hypersecretion in a dose-dependent way. The symptoms appear instantaneously after serotonin application. Earlier, we found no change in nasal airway resistance following serotonin provocation (17), and in the present study the volunteers reported only a slight feeling of nasal blockage. Topical pretreatment with atropine partially inhibited the nasal secretory response to serotonin, probably in a competitive fashion. Unilateral challenge with serotonin produced small amounts of secretions on the contralateral side. These findings suggest that serotonin-induced nasal symptoms are partly reflex-mediated (9). It is understandable that atropine did not affect the number of sneezes, as this symptom is initiated by afferent signals to the central nervous system.

Pretreatment with anti-histamine and anti-serotonin drugs did not influence the serotonin-induced nasal response at all. Even though topical pretreatment had no effect, one cannot totally exclude a possible effect of the drug: the concentration of the drug may have been too low, the penetration of the drug to the site of action may be poor, or the disappearance of the drug may be too rapid. On the other hand, in other studies (8, 11, 12) topical pretreatment with anti-histaminic drugs, including chlorpheni-

ramine in the same concentration as used by us, has inhibited the nasal response to exogenous histamine. Topical anti-cholinergics, including atropine, inhibit the nasal response to methacholine challenge but not to allergen (8, 10, 12), while intramuscular administration of atropine inhibits the allergen challenge (4). In 18 house dust-allergic patients nasal pretreatment with methysergide (0.5 mg/ml) did not show any protective effect on allergen provocation (12). In a double-blind study there was no difference between oral treatment with cyproheptadine and chlorpheniramine in their effect on symptoms of hay fever in 35 grass pollen-allergic rhinitis patients (15). According to this, anti-serotonin drugs seem to have no effect in allergen-induced rhinitis. This is in agreement with the present observation that pretreatment with methysergide did not inhibit the effect of serotonin intranasally. We have found that serotonin-induced nasal secretion is partly reflex-mediated, acting on afferent sensory nerve endings in and beneath the epithelium (14). It has been proposed that histamine acts on specific H_1 -receptors on these nerves. As anti-histaminics did not influence the effect of serotonin in this study, serotonin may act on specific serotonergic receptors.

Based on binding studies of the brain, serotonergic receptors can be divided into serotonin- S_1 and S_2 receptors (3). Serotonin- S_1 and S_2 -receptors are characterized by high-affinity binding for serotonin and serotonin antagonists (spiperone and ketanserin), respectively (5, 13, 19). Only the S_2 -receptors have been shown to mediate various pharmacological effects, and have primarily been found located to the brain (6). Peripheral S_2 -receptors have been demonstrated on platelets (7). There is indirect pharmacological evidence that S_2 -receptors are present also in the smooth muscle of the blood vessel wall and in the bronchial tissue (2, 18), but the presence of serotonergic receptors in the nose is only hypothetical.

The reproducibility of the serotonin nasal challenge was fairly good, especially regarding the amount of secretions, and can be compared with the reproducibility of the histamine and methacholine nasal provocation test (1, 20). The amount of secretion was greater than in a recent

study of nasal challenge with serotonin done by us (17). This may reflect the great individual difference in nasal response to serotonin and various other nasal stimuli and also justifies the use of a cross-over design in the present study.

Serotonin is found in nasal secretions after histamine and methacholine nasal challenge in concentrations 10^2 – 10^4 nmol/l (16), but the concentration used for challenge was about 10^3 – 10^4 times greater. The difference in provoking histamine concentration and the concentration of histamine in nasal secretions is also about 10^4 times. This might indicate a clinical importance for serotonin. The actual concentration of the challenge drug in the nose is probably diminished because of an unknown degree of dilution when dissolved in the mucous layer.

In summary, nasal discharge contains serotonin, and challenge by serotonin induces nasal secretions, in part reflex-mediated. Serotonin might therefore play a physiological role in rhinitis and other conditions with nasal secretion.

REFERENCES

1. Borum, P.: Nasal methacholine challenge. *J. Allergy Clin. Immunol.* **63**, 253–257, 1979.
2. Eccles, N. K., Grimmer, A. J. & Leathard, H. L.: Action of ketanserin on human isolated blood vessels. *Br. J. Pharmacol.* **74**, 831–835, 1981.
3. Ilien, G., Gorrison, H. & Laduron, P. M.: Characterization of solubilized serotonin (S_2) receptors in rat brain. *Mol. Pharmacol.* **22**, 243–249, 1982.
4. Konno, A., Togawa, K. & Fujiwara, T.: Mechanisms involved in onset of allergic manifestations in the nose. *Eur. J. Respir. Dis.* **64**, Suppl. 128, 155–166, 1983.
5. Leysen, J. E., Awouters, F., Kenis, L., et al.: Receptor binding profile of R 41 468, a novel antagonist at 5-HT receptors. *Life Sci.* **38**, 1015–1019, 1981.
6. Leysen, J. E., Geerts, R., Gommeren, W., Verwings, M. & Van Gompel, P.: Regional distribution of serotonin-2-receptor binding sites in the brain and effects of neuronal lesions. *Arch. Int. Pharmacodyn. Ther.* **156**, 301–305, 1982.
7. Leysen, J. E., Gommeren, W. & De Clarck, P.: Demonstration of S_2 -receptor binding sites on cat blood platelets using [^{3} H] ketanserin. *Eur. J. Pharmacol.* **88**, 125–130, 1983.
8. McLean, J. A., Mathews, K. P. M., Solomon, W. R., Brayton, P. R. & Ciarkowski, A. A.: Effect of histamine and methacholine on nasal airway resistance in atopic and nonatopic subjects. *J. Allergy Clin. Immunol.* **59**, 165–170, 1977.
9. Mygind, N.: Mediators of nasal allergy. *J. Allergy Clin. Immunol.* **70**, 149–159, 1982.
10. Mygind, N. & Borum, P.: Effect of a cholinoreceptor antagonist in the nose. *Eur. J. Respir. Dis.* **64**, Suppl. 128, 167–174, 1983.
11. Mygind, N., Secher, C. & Kirkegaard, J.: Role of histamine and antihistamine in the nose. *Eur. J. Respir. Dis.* **64**, Suppl. 128, 16–20, 1983.
12. Pelikan, Z. & De Vries, K.: Effects of some drugs applied topically to the nasal mucosa before provocation tests with allergen. *Acta Allergol.* **29**, 337–353, 1974.
13. Peroutka, S. J. & Snyder, S. H.: Multiple serotonin receptors: Differential binding of [^{3} H] 5-hydroxytryptamine, [^{3} H] lysergic acid diethylamide and [^{3} H] spiroperidol. *Mol. Pharmacol.* **16**, 687–699, 1979.
14. Secher, C., Kirkegaard, J., Borum, P., Maansson, A., Osterhammel, P. & Mygind, N.: Significance of H_1 and H_2 receptors in the human nose: rationale for topical use of combined antihistamine preparations. *J. Allergy Clin. Immunol.* **70**, 211–218, 1982.
15. Smellie, H. & Fry, L.: Comparison of an antiserotonin (cyproheptadine) and a pure antihistamine (chlorpheniramine) in hay fever. *Acta Allergol.* **17**, 352–357, 1962.
16. Tønnesen, P. & Hindberg, I.: Serotonin in histamine and methacholine induced nasal secretions. (In preparation).
17. Tønnesen, P. & Mygind, N.: Nasal challenge with serotonin and histamine in normal persons. *Allergy* **40**, 350–353, 1985.
18. Van Nueten, J. M., Janssen, P. A. J., De Ridder, W. & Vanhoutte, P.: Interaction between 5-hydroxytryptamine and other vasoconstrictor substances in the isolated femoral artery of the rabbit; effect of ketanserin. *Eur. J. Pharmacol.* **77**, 281–287, 1982.
19. Van Nueten, J. M., Leysen, J. E., Schuurkes, J. A. J. & Vanhoutte, P.: Ketanserin: a selective antagonist of 5-HT $_2$ serotonergic receptors. *Lancet* **1**, 279–298, 1983.
20. Wihl, J.: Methods for assessing nasal reactivity. *Eur. J. Respir. Dis.* **64**, Suppl. 128, 175–179, 1983.

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