

Nasal challenge with serotonin in asymptomatic hay fever patients

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To study the nasal response to serotonin, 14 asymptomatic hay fever patients received intranasal serotonin in increasing doses. Itching and the number of sneezes were noted, and the amount of secretion was measured and assayed for substance P. Nasal airway resistance was recorded by active posterior rhinomanometry. Serotonin induced a dose-dependent increase in nasal itching, number of sneezes and secretion. Substance P was found in 41 of 42 secretions. The median concentration was 10.3 pmol/l (range 0-28.9), and increased parallel to increasing doses of serotonin ($P < 0.001$).

Key words: rhinitis; serotonin; substance P.

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CLINICAL ASPECTS

Serotonin nasal challenge with increasing doses was performed in 14 grass-pollen allergic hay fever patients. Serotonin induced a dose-dependent response, i.e. itching, sneezes and secretion. The nasal airway resistance did not change. All secretions but one contained substance P. Further investigations of substance P in nasal secretions in humans should be made.

We have previously reported that nasal challenge with serotonin induces itching, sneezes and secretion in normal subjects (12). The response increased parallel to the applied serotonin dose. Topical pretreatment with atropine reduced the volume of secretion after serotonin challenge, while an anti-serotonin drug had no effect. Unilateral nasal challenge with serotonin induced bilateral secretion (13). These findings suggest that serotonin acts on sensory nerves with reflex-induced sneezes and hypersecretion. Thus, serotonin might play a role in the normal nasal secretory response. This is further supported by the finding of serotonin in nasal secretions induced by histamine, methacholine and allergen (14).

Serotonin-induced nasal secretions in normal subjects also contain substance P (SP) (15). This agent is found in the afferent, sensory nerves in the nose, and might be released when

these nerves are activated for instance by serotonin. SP is one of the most potent agents to stimulate secretion from tracheal glands in animals *in vitro* (2). However, in the human nose *in vivo* topical challenge with SP had no effect on nasal secretion (8) nor on mucociliary function (6).

The aim of the present study is to examine the effect of nasal serotonin challenge in hay fever patients and to examine the effect on concentration of SP in nasal discharge.

MATERIAL AND METHODS

Patients

Fourteen grass pollen-allergic hay fever patients were examined in an asymptomatic period during the month of February. They used no daily

medicine up to the date of investigation. All had free air passage through both nostrils. Only one suffered from asthma. All had positive skin prick test and positive nasal challenge with grass pollen (timothy). All suffered from rhinitis in June-July, four had also birch and three mugwort allergy. Except one, who smoked 10 cigarettes daily, all were non-smokers. Eight were female and six male with a median age of 23 years (range 20-28).

Provoking agent

Serotonin creatinine sulphate (serotonin) (MW: 380) in a phosphate buffer was used in three concentrations, 0.8 mg/ml, 4.0 mg/ml and 20.0 mg/ml; pH was 7.40, 7.10 and 6.08, respectively. The osmolality was 240 mOsm. The solutions for nasal provocation were filled in metered-dose pump sprays, which delivered 0.065 ml and 0.050 ml per actuation, respectively.

Nasal challenge

Each provocation was started with a control challenge with a phosphate buffer (pH 7.42; 240 mOsm), one puff into each nostril. Four increasing doses of serotonin were then given with 15 min intervals, one puff into each nostril, except for the last dose where 2 puffs × 2 were used. The provocative doses of serotonin were 104 µg, 520 µg, 2600 µg, 4000 µg, respectively.

Nasal airway resistance (NAR) was assessed by active posterior rhinomanometry (11, 12). Measurement of NAR was performed in duplicate immediately before and 5 min after each step in the nasal challenge procedure. The secretions were collected in a 5-min period after provocation: the patient bent his head forward, and the drips were collected into a funnel. At the end of the period the patient blew his nose to collect retained secretion. The patient graded itching (grade 0-10), and counted the number of sneezes in the same 5-min period after challenge. Patients were asked about any adverse symptoms such as heating, flushing and headache.

Substance P

The secretions were weighed immediately after collection and stored at -80°C within maximum 5 min. SP was assayed by radioimmunoassay after ethanol extraction as described elsewhere (15).

Statistical methods

Mann Whitney and Wilcoxon's matched-pairs signed-ranks tests were applied, and a *P* value ≤ 0.05 was considered statistically significant.

RESULTS

No significant changes in NAR was observed after serotonin challenge (Table 1). An increase

Table 1
Results of nasal challenge with increasing serotonin doses compared to buffer in 14 hay fever patients

Provocative agent	Buffer	Serotonin			
		104 µg	520 µg	2,600 µg	4,000 µg
NAR	35.8 (35-45)	40.8 (26-47)	39.1 (30-46)	40.5 (29-53)	41.3 (30-79)
No. of sneezes	0 — * * — 1.5 (0)	0-6	2.5 (0-5)	2.5 — * * — 1.0 (0-8)	1.0 (0-8)
Itching (grade 0-10)	0 — * — 2.0 (0-2)	0-6	3.0 — * * — 4.5 (0-5)	4.5 (2-8)	4.5 (0-8)
Secretion (mg)	0 (0-965)	131 — * * — 386 (0-872)	386 — * — 668 (0-1329)	668 (50-2374)	744 (50-2650)

NAR = nasal airway resistance. * *P* < 0.05; ** *P* < 0.01

of 14.0 % in NAR was found after the lowest serotonin dose, but only a 1.2 % increase in NAR was found from the lowest to the highest serotonin dose. Five patients (36 %) had an increase in NAR above 30 % (35 %, 37 %, 51 %, 59 % and 104 %).

Itching and secretion volume increased in a dose-related manner (Table 1), but the number of sneezes and itching did not increase further after the serotonin dose, 2600 µg, whereas the number of sneezes was found to decrease with 4000 µg serotonin.

SP was found in all but one of the secretions (Table 2). The median concentration was 10.3 pmol/l (range 0–28.9). The concentration of SP increased in a dose-related manner in response to larger serotonin doses (55 %, 80 % and 100 % of the SP concentrations compared to 104 µg serotonin) (Spearman test, $R = 0.418$, $P < 0.001$). But no significant increase was found from one dose to the next dose. However, when considering the absolute amount of SP (volume × concentration of SP), there was a significant difference from one dose to the next, except between the last doses (2600 µg to 4000 µg).

Four subjects (29 %) had no side effects after serotonin challenge. Seven (50 %) reported itching of the throat and mouth, six (43 %) of the

palate and one (7 %) of the eyes. The symptoms were identical to those experienced during hay fever attacks in the summer. Three (21 %) developed hyperesthesia of the upper lip and upper, frontal teeth.

DISCUSSION

The nasal response elicited by serotonin consisted of itching, sneezes and secretion. A dose-response relationship was observed for all these symptoms with the usual S-shape. Another explanation could be tachyphylaxis in response to the highest serotonin dose for sneezing and to a lesser degree for itching. Tachyphylaxis is well known with both serotonin and histamine (4, 11). However, the concentration of serotonin used must be considered a pharmacological dose. NAR did not change significantly in this trial, but in three hay fever patients (21 %) NAR increased more than 50 %. These three patients did not otherwise differ from the rest of the group. Because serotonin had no overall effect on NAR, any important effect on capacitance vessels seems unlikely, in contrast to the changes during an allergen-induced hay fever attack. However, the hay fever patients complained of symptoms of the mouth and throat, compatible with their symptoms during a normal hay fever attack.

We have previously performed similar serotonin nasal challenge experiments in normal subjects (12, 13). Hay fever patients appear to be no more hyperreactive to serotonin, than normal subjects. Increased nasal reactivity to histamine and methacholine has been reported in rhinitis patients (1, 3, 5, 10), but not in pollen-allergic patients outside the pollen season (7, 9), which is in agreement with the present findings with serotonin.

SP was found in almost all of the secretions, and a dose-response relationship was found between serotonin dose and the concentration of SP in hay fever patients. This has also been observed in normal subjects (15). The concentration of SP in nasal discharge did not differ between hay fever patients and normal subjects (15). From this and earlier findings the following hypothesis can be proposed: Nasal

Table 2
Concentration of substance P (pmol/l) in serotonin-induced nasal secretions in 13 hay fever patients

No.	Provocative serotonin dose			
	104 µg	520 µg	2600 µg	4000 µg
1		10.3	15.5	17.6
2		17.2	11.7	17.6
3	5.8	8.5	14.0	16.0
4			0	28.9
5	8.3	11.0	15.5	13.0
6	10.5	18.0	22.5	10.5
7				17.4
8	6.5	3.0	7.5	11.0
9	5.8	7.1	8.5	12.5
10			12.0	13.0
11	5.5	8.0	6.5	13.0
12		10.0	9.8	16.0
13	11.4	10.1	13.6	7.5
Median	6.5	10.1	11.7	13.0
Range	(5.5–11.4)	(3.0–18.0)	(0–22.5)	(7.5–28.9)

stimulation by serotonin for instance might act on the sensory afferent nerves in the nasal mucosa and activate an afferent reflex arch, resulting in secretion, itching and sneezes. When the afferent nerves are activated, SP is released and acts on glands in the mucosa. Thus, nasal stimulation might also release serotonin into the nasal secretions. The released serotonin might amplify the nasal response by acting on the afferent neurons. This fits with our findings that atropine pretreatment can only partly reduce the secretion after nasal challenge (the reflex-induced secretion). The remaining secretion might then be induced by released SP (15). Atropine resistance is a characteristic feature of regulatory peptides such as SP (2). SP was found to be one of the most potent secretory agents *in vitro* in animal tracheal glands (2). However, nasal challenge with SP in human subjects did not elicit any secretion, although a subjective impression of secretion was reported (8). Thus further studies in man are necessary to confirm the above hypothesis of SP as a secretory agent in man.

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