

Serotonin in nasal secretion

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The aim of this study was to investigate the serotonin content in nasal secretion. Histamine- and methacholine-induced nasal secretions were collected during a 5-min period after challenge in 33 normal subjects. Serotonin was determined radioenzymatically and was found in 66% of pure, blown secretions. The median concentration of serotonin in histamine- and methacholine-induced secretions was 6.3 nmol/l (range < 1.6-52.0) and 2.3 nmol/l (range 1.6-140.0) (NS), respectively. The serotonin concentration in allergen-induced nasal secretions in 7 rhinitis patients was 0.8 to 159 nmol/l, median 10.4 nmol/l which did not differ from the serotonin levels in the normal subjects. The serotonin concentration in nasal lavage fluids in 5 subjects was low (below the detection limit in 78% of the samples). Further studies of serotonin in patients with nasal hypersecretion are indicated.

Key words: histamine; methacholine; nasal challenge; nasal secretion; serotonin.

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In preliminary investigations we have found serotonin in nasal secretions (19). The role of serotonin in nasal secretion and rhinitis is almost unknown. However, nasal challenge with serotonin in supraphysiological doses elicits sneezes, itching and hypersecretion in both normal subjects and rhinitis patients (17, 21, 23). Intradermal injections of serotonin in a physiological dose produces a triple response and induces constriction of the subcutaneous veins (18). During allergen-induced rhinitis an immediate increase in plasma serotonin was observed (20). This does not prove that serotonin is a mediator in rhinitis, although some of its effects fit with intranasal events during a rhinitis response.

Agents with a possible role in rhinitis, such as histamine, kinins, leukotrienes, substance P, and vasoactive intestinal peptide, have been measured in nasal secretions (4, 7, 12, 15, 22). No data about serotonin in nasal discharge have been published, but 5-hydroxyindolyl acetic acid, the main metabolite of serotonin, has been found in sputum of asthmatics (10).

In this study we examined the applicability of a radioenzymatic serotonin assay on nasal secre-

tions. We report serotonin concentrations in histamine, methacholine and allergen-induced secretions.

MATERIAL AND METHODS

Study design

Methacholine and histamine were used because of their differing effect on secretion (11): methacholine stimulates glands directly, while histamine also increases plasma transudation. The doses chosen were the "maximal" for use without causing side effects, and in preliminary experiments these doses evoked about the same volume of secretion.

Methacholine and histamine challenges were performed on 2 separate days in 33 subjects: one with 0.5 mg histamine, and one with two doses of methacholine 5 mg ($n = 16$), followed after 5 min with 10 mg ($n = 33$).

Allergen challenge was performed in 7 hay fever patients with one or two doses of timothy, depending on the reaction. Nasal lavage was performed in 5 normal subjects before and after

control challenge, and after histamine challenge with 10-min intervals with 0.02 mg, 0.1 mg and 0.5 mg histamine. On a separate day methacholine challenge was done in the same way with 1.0 mg, 5.0 mg and 20.0 mg.

Day-to-day variation in serotonin concentration was estimated in subjects by histamine challenge with 0.2 ml of histamine, 2.5 mg/ml, with a week's interval.

Subjects

Thirty-one women and 13 men, aged 17–50 years (median 33 years) volunteered to participate. All were healthy, non-atopics with no upper respiratory tract infections during the last 4 weeks, all had free nasal passage and none was using daily medicine. Informed consent was obtained after both verbal and written information.

Seven hay fever patients (3 women and 4 men) with timothy grass pollen allergy were provoked with timothy outside the grass pollen season. Their median age was 26 years (range 19–37). None suffered from rhinitis symptoms during the study nor used any medicine.

Provocative agents

Provocative agents used were histamine chloride (histamine), 0.1, 0.5, 2.5 mg/ml in phosphate-buffered saline (PBS) pH 7.1, and methacholine bromide (methacholine), 5.0, 25, 50, 100 mg/ml in PBS, pH 7.4. No preservatives were added. PBS was used for control challenge. Nasal lavage was performed with isotonic saline. For allergen challenge we used dialysed timothy grass pollen extract (Aquagen SQ, ALK, Copenhagen), supplied as a freeze-dried stock solution (100,000 SQ Units/ml). Solutions of 1,000, 10,000 or 100,000 SQ Units/ml were used for provocation.

Nasal challenge

The two provocation tests were carried out in random order at the same time of day with an 8-day interval. Each provocation began with a control challenge with PBS, 1 puff into each

nostril, using a pumpspray delivering 100 µl/actuation. After 5 min either histamine or methacholine was administered, 1 puff into each nostril, the subject simultaneously inhaling. In the 5-min period after provocation the person bent his head forward, and after 5 min blew the secretion into a funnel with a plastic syringe attached. The secretions were weighed and stored at -20°C within 5 min. The hay fever patients were provoked with timothy solution, 0.1 ml bilaterally. A provocation was considered positive when more than 0.4 ml of secretion was obtained and the nasal peak flow decreased more than 20%. The initial allergen dose depended on the skin reaction to timothy. If the nasal reaction was not positive after 5 min a 10-fold higher dose was given.

Specimens for microscopy

After the histamine-induced nasal secretions were collected, a cotton bud was gently inserted into the left nasal cavity and rolled against the nasal floor and septum. A smear was then made and stained with Giemsa. The specimens were examined by light microscopy for platelets, erythrocytes and leucocytes. Also pure secretions were examined.

Nasal washing

The lavage method is described by Bisgaard et al. (2). A 5-cm long soft polyethylene catheter (Venflon, Viggo) was inserted along the septum below the inferior turbinate. The subject bent his head forward, 1 ml of isotonic saline was instilled and left in the nasal cavity while the subject gently occluded the vestibulum of the nose. After 30 s the subject blew his nose into a funnel. The procedure was repeated in the opposite nostril. The lavage with 1.0 ml saline was repeated twice with 1 min interval, followed by challenges with a control solution (NaCl-1, NaCl-2). Pure secretion was collected in a 10-min period by spontaneous dripping terminated by blowing the nose, followed by lavage with 1.0 ml saline bilaterally. Repeat challenges with 3 increasing doses of histamine or methacholine were then performed as above.

Serotonin in nasal secretion

Nasal lavage fluids were analysed without dilution while pure nasal secretions were diluted with equal amounts of PBS containing albumin, 80 g/l, added 2–3 glassbeads and shaken for 20 min to homogenize the secretions. Determination of serotonin was made by radioenzymatic methods as described by Hindberg (5). In this assay the sample is acetylated with acetic acid anhydride and further methylated with S-adenosyl-L-([³H]methyl)-methionine ([³H]SAM) and the enzyme hydroxyindole-O-methyltransferase (HIOMT). The [³H]melatonin formed is extracted with toluene and the radioactivity is determined after washing and re-extraction of the toluene extract. The detection limit (defined as the concentration giving rise to radioactive counts of 3 × the SD of a blank) is 0.8 nmol/l corresponding to 1.6 nmol/l in pure nasal secretions. The inter-assay relative SD is 0.11 (11%). HIOMT is specific for hydroxy indoles, and thin layer chromatography in three different solvents showed identical *R_f*-values for melatonin and the radioactive product formed in the assay of serotonin in the nasal secretions (5). Further, linearity was found when serial dilutions of nasal secretions with high serotonin concentrations were analysed using the buffer for plasma analyses as diluent.

Statistics

Wilcoxon's matched-pairs test was used to compare serotonin concentrations and volume of secretion obtained with different challenges (histamine vs. methacholine, low dose vs. high dose of methacholine). *P* values below 0.05 were considered significant. Median and range were used as descriptive statistics.

RESULTS

Pure secretion

No secretion was obtained after control challenge. The amount of histamine-induced secretion was 0.27 g (median), (range 0.01–0.99) (*n* = 33), and

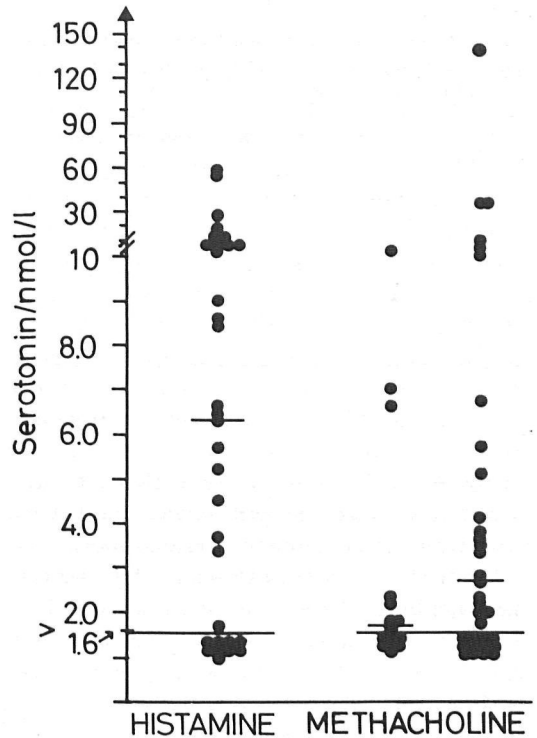


Fig. 1. Serotonin concentrations in nasal secretions obtained from normal subjects provoked with histamine 0.5 mg (*n* = 33), and methacholine, 5 mg (*n* = 16) and 10 mg (*n* = 33). Vertical bars: median values. Differences are not significant.

that of the methacholine-induced was 0.25 g (range 0.05–0.58) (*n* = 16) after the low methacholine dose and 0.28 g (range 0.11–1.32) (*n* = 33) after the high dose. There was no significant difference in secretion amount. However, for the 16 paired secretions after the two doses of methacholine, the median amount was 0.25 g for the low dose and 0.37 g for the high dose (*P* < 0.05).

Serotonin was detected in 66% of the secretions. The median serotonin concentrations in secretions induced by histamine and methacholine were 6.3 nmol/l and 2.3 nmol/l (NS), respectively (Fig. 1). In methacholine-induced paired secretions (low and high doses) the concentration of serotonin was not statistically different. The amount of secretion did not correlate with serotonin concentration. The day-to-day variation of serotonin concentration was

Table 1

Day-to-day variation in serotonin concentrations in histamine-induced nasal secretions in 6 normal subjects. Histamine concentrations, 2.5 mg/ml; dose 0.5 mg

Subject No.	Serotonin concentration/nmol/l	
	Day 1	Day 8
1	< 1.6	< 1.6
2	55	40
3	< 1.6	< 1.6
4	< 1.6	< 1.6
5	64	35
6	3.2	4.1

relatively small (Table 1). Re-analysis of samples after days and several months showed no significant fall in serotonin concentrations.

The median volume of secretion after allergen challenge in the 7 hay fever patients was 0.85 g

Table 2

Serotonin concentrations (nmol/l) in timothy grass pollen-induced nasal secretions in hay fever patients

Subject No.	Serotonin/nmol/l			
	SQ units/ml:	1,000	10,000	100,000
1		62	< 2.4	-
2		-	159	24.5
3		-	7.2	-
4		-	121	46.2
5		-	4.5	-
6		10.4	-	-
7		-	4.8	-

Median concentration: 10.4 nmol/l

(range 0.4–1.2). Serotonin was found in all 10 secretions (Table 2). The median serotonin concentration was 10.4 nmol/l, and was not significantly different from the concentrations found in histamine- and methacholine-induced secretions.

Table 3

Concentration of serotonin (nmol/l) in nasal lavage fluid (lav) and pure blow secretions (sec) in 5 normal subjects after nasal challenge with isotonic sodium chloride, methacholine and histamine. Nasal washing volume 1.0 ml bilaterally

Provocation agents		Subjects				
		1	2	3	4	5
NaCl-1	(lav)	< 0.8	< 0.8	1.2	< 0.8	1.6
NaCl-2	(lav)	0.9	< 0.8	< 0.8	< 0.8	1.4
Methacholine 5 mg/ml	(sec)	-	-	-	-	5.4
	(lav)	< 0.8	< 0.8	< 1.2	2.2	2.4
Methacholine 25 mg/ml	(sec)	1.5	< 1.4	< 1.8	-	1.9
	(lav)	< 0.8	< 0.8	< 0.8	< 0.8	4.0
Methacholine 100 mg/ml	(sec)	< 1.6	< 1.5	< 1.6	-	1.6
	(lav)	< 0.8	< 0.8	< 1.3	2.5	2.8
NaCl-1	(lav)	< 0.8	< 0.8		< 0.8	1.1
NaCl-2	(lav)	< 0.8	< 0.8		< 0.8	< 0.8
Histamine 0.1 mg/ml	(sec)	< 1.6	-		-	-
	(lav)	< 0.8	< 0.8		< 0.8	< 0.8
Histamine 0.5 mg/ml	(sec)	-	-		-	-
	(lav)	< 0.8	< 0.8		< 0.8	< 0.8
Histamine 2.5 mg/ml	(sec)	< 1.7	< 1.4		22.1	< 1.6
	(lav)	< 0.8	< 0.8		< 0.8	< 0.8

Microscopy of the 12 nasal smears and pure secretions revealed no platelets or erythrocytes in any of the specimens, but many leukocytes, bacteria and squamous and cuboidal epithelial cells.

Nasal lavage fluid

The median recovery of fluid after lavage with 1.0 ml bilaterally after control challenge was 91 % (range 72 %–111 %) ($n = 16$). After histamine challenge the median recovery was 96 % and after methacholine 104 %. The basal concentration of serotonin in lavage fluid from the “unprovoked” nose ($n = 9$) and after control challenge with isotonic saline was 1.6 nmol/l or below the detection limit. The concentration was below the detection limit of the assay in 78 % (42/54) of the samples, and the highest value was 4.0 nmol/l. The highest serotonin concentration in pure secretion was 22.1 nmol/l, with no detectable serotonin in the lavage fluid just after the pure secretion was blown (Table 3).

DISCUSSION

This is the first study dealing with serotonin in nasal secretions. The radio-enzymatic method used is highly specific for serotonin (5). Chromatography of the end-product of the analysis strongly supports that the nasal discharge contained “authentic” serotonin.

Serotonin concentrations in the secretions varied with a factor 100, which is less than the variation reported for histamine (4, 7). The cholinceptor agonist, methacholine, stimulates glands directly and does not affect nasal resistance, while histamine also induces plasma transudation and affects nerves and mast cells in the mucosa, thus resulting in mucosal swelling and fall in nasal peak flow. Even though the contribution of plasma transudation differs after methacholine and histamine challenge (3), the serotonin concentrations did not differ after these two agents. This suggests that plasma transudate is not the sole source of serotonin in nasal secretions.

The nasal lavage method was applied because

it was not possible to obtain pure blown secretions after control challenge. It showed that the basal and placebo-induced concentration of serotonin in the nasal cavity is low. However, by this method the secretions were so diluted that in most samples the concentration of serotonin was below the assay limit. Thus we do not know if the serotonin level in stimulated secretion was increased, as serotonin was only found in a few of the lavage fluids after stimulation. The pure secretion was blown prior to lavage, which supports that only little serotonin is retained in the nasal cavity after 10 min. As serotonin was found in only 20 % of the samples, nasal lavage does not seem to be a suitable method for measuring serotonin in secretion unless the lavage fluid is reduced. The recovery of the nasal lavage fluid equalled that described by Bisgaard et al. (2).

The serotonin levels found in the secretions from the 7 hay fever patients did not differ from the findings in normal subjects. Concentrations tended to be higher after the first allergen dose than after the second. However, the number of subjects was too low to confirm this. In 14 rhinitis patients the median concentration of serotonin in allergen- and methacholine-induced secretions was 7.2 nmol/l and 3.7 nmol/l, (NS) respectively (20). These concentrations do not differ from the results in normals in the present report.

The observed serotonin levels in the secretions are in the same range or higher than in plasma. As only a part of the secretion is plasma transudate (1, 7, 14), this does not seem to be the sole source of serotonin, in accordance also with the observation of no difference in serotonin concentrations in histamine- and methacholine-provoked secretions. During allergen-induced rhinitis we found an immediate increase in plasma serotonin in venous blood, and this small increase might reflect even higher increases locally in the nasal mucosa. Because there was no difference in the serotonin level in nasal discharge after allergen and histamine and methacholine challenges, one should also expect an increase in plasma serotonin during methacholine and histamine challenge. This has to be evaluated.

Human mast cells do not contain serotonin (13, 16), and, in fact, platelets are the only human blood cells containing serotonin. We found no platelets or red blood cells in the nasal specimens. The concentration of serotonin in 10^9 platelets is approximately 5 nmol (6). A contribution of only 0.5 nmol/l in the nasal secretions would require 10^8 platelets. This makes it unlikely that high amounts of serotonin have been released from platelets *in vitro*.

Using serotonin antibodies we have found only a single serotonin-containing cell in several biopsies from the nasal mucosa in man. Using the same method we have localized many serotonin-containing cells in the trachea and bronchi in man and pig (Poulsen, S. S. & Tønnesen, P., unpublished observations). The role of these neuroepithelial cells in bronchial tree epithelium is unknown (8, 9). Further studies are needed to evaluate to what extent serotonin-containing cells are present in the nasal mucosa and if they significantly contribute to serotonin in nasal secretion.

In conclusion, histamine-, methacholine- and allergen-induced nasal secretions contain serotonin. This does not prove a connection between serotonin and rhinitis, but it leaves the possibility open.

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