

Effect of nasal allergen challenge on serotonin, substance P and vasoactive intestinal peptide in plasma and nasal secretions

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We have studied the changes in concentration of serotonin, substance P, and vasoactive intestinal peptide (VIP) in plasma following a nasal allergen provocation in 14 grass pollen-allergic subjects; in five the urinary excretion of serotonin and 5-hydroxy-indolylacetic acid (5-HIAA) was also measured. In addition, the concentration of serotonin and substance P was measured in nasal secretions following nasal challenge with allergen and methacholine. The results showed an allergen-induced increase in free plasma serotonin ($P < 0.01$) and no change in platelet serotonin, urinary serotonin and urinary 5-HIAA. The plasma substance P level tended to fall ($P > 0.1$), while plasma VIP increased significantly ($P < 0.02$). In nasal secretions, there were measurable levels of serotonin in all samples and of substance P in all but one. There was no difference between the concentrations of serotonin and substance P in secretions collected after allergen challenge and after methacholine challenge. For both substances, the secretion median value was comparable to that of plasma. Symptom reduction by topical and systemic pretreatment with a serotonin- and VIP-antagonist before nasal allergen provocation is necessary to define the role of these two agents in allergic rhinitis.

Key words: allergic rhinitis; nasal allergen provocation; serotonin; substance P; vasoactive intestinal peptide

Accepted for publication 11 February 1988

Histamine has for many years been considered the most important biochemical mediator of allergic rhinitis, in agreement with the marked effect of antihistamines in the treatment of this disease (19, 37). However, it is likely that histamine acts in concert with a number of other mediator substances, which can be: 1) mast cell-derived (e.g. leukotriene); 2) derived from other cell types in tissue or blood (e.g. eosinophil cationic protein and serotonin); 3) released from nerves (e.g. substance P and VIP) (21, 22).

The relative importance of the single biochemical mediator can be analysed in different ways: 1) by nasal allergen provocation and measurement of the potential mediator substance in plasma and, probably better, in nasal

secretions; 2) by nasal provocation test with the substance in question; 3) by a study of the efficacy of specific antagonists in allergic rhinitis.

The present study deals with serotonin, substance P and VIP which all have the potential of being significant mediators of nasal allergy. Serotonin can, by mast cell-derived platelet activating factor, be released from platelets (5, 7) and probably also from cells in the bronchial epithelium (10, 12); in the human nose, it causes marked itching, sneezing and hypersecretion (29, 31). Substance P increases vascular permeability and stimulates exocrine glands in animals (11, 15, 16) although not with certainty in man (17). It is released from sensory nerve endings and has been demonstrated in

the human nose (14, 33). VIP, which increases vascular permeability (18, 39), is released from the parasympathetic nerve terminal, also in the human nose (13, 32). We have measured all 3 substances in plasma but only serotonin and substance P in nasal secretions, as a pilot study disclosed very low or immeasurable levels of VIP.

MATERIAL AND METHODS

The investigation comprises three parts: 1) measurement of serotonin, VIP and SP in plasma after nasal allergen provocation; 2) measurement of serotonin and its metabolite 5-HIAA in urine after nasal provocation; 3) measurement of serotonin and substance P in nasal secretions collected after provocation with allergen and with methacholine.

Volunteers

Fourteen asymptomatic hay fever patients volunteered to take part in this study (4 women and 10 men). Their mean age was 24 years (range 18 to 35). All had rhinitis symptoms in June and July and a positive skin prick test and nasal provocation test to timothy grass pollen. The study was undertaken in March and April. None suffered from asthma or perennial rhinitis, and rhinoscopy did not reveal any gross anatomic abnormalities. None received medication. Informed consent was obtained after both verbal and written information. The study protocol was approved by the Copenhagen Committee on Ethics. Only 5 of the volunteers participated in part 2 of the study.

Nasal allergen challenge

Allergen extract. Dialysed timothy grass pollen extract was used (Aquagen SQ, ALK, Copenhagen). It was supplied as a freeze-dried stock solution (100,000 SQ units/ml). Ampules, all from the same batch, were stored at 5°C and kept at room temperature for 1 h before provocation.

Pre-trial. One month before the trial all volunteers were provoked with increasing allergen

doses in order to establish the individual threshold dose, which was defined as the lowest dose causing 0.4 ml of secretion and 20% reduction of nasal peak expiratory flow. Using a DeVilbiss Atomizer No. 15, 0.1 ml was given in each nostril while the volunteer held his breath. The provocation started with an allergen concentration of 100 SQ Units/ml, increasing step-wise every 10 min with increments of a factor 10 until positive reaction or the concentration of 100,000 SQ Units/ml was reached.

Trial. On the test day, the volunteers came to the laboratory at 9 a.m. and rested for 20 min. Testing was started by spraying isotonic saline into the nose; 15 min later the individual threshold dose was administered as a single dose.

Sampling of nasal secretions. Immediately after the allergen provocation, the volunteer bent his head forward and remained in this position for 5 min, dripping secretion into a funnel attached to a syringe and then blowing the nose at the end of this period. The samples were placed in the freezer and kept at -20°C until analysis.

Symptom recording. The volume of secretion was measured. The number of sneezes was counted, and the volunteers gave a score (0-10) for immediate itching in the nose. Using a Wright mini-peak flow meter with an anaesthetic face mask attached, the nasal peak expiratory flow was measured (highest of 3 recordings) immediately before and 5, 15, and 30 min after allergen provocation.

Nasal methacholine provocation

Allergen and methacholine provocations were carried out in a random order with an interval of 8 days. Methacholine bromide (Sigma) (10 mg) was used for provocation. The spraying technique, symptom recording and sampling method for secretions were the same as for allergen challenge.

Blood sampling

A short plastic catheter (Venflon, Viggo) was inserted into a cubital vein. Blood samples were

drawn before and 5 and 15 min after nasal challenge with saline and 2, 5, 15, and 30 min after allergen challenge. The catheter was kept open by slow infusion of isotonic saline, and at each blood sampling, the first 10 ml blood was discharged.

Serotonin in platelet-poor plasma. Nine ml blood was drawn into an ice-chilled polypropylene tube containing 1 ml citrate solution and placed in an ice-bath for 10–20 min. After centrifugation at 4°C at 180 *g* for 15 min, the platelet-rich plasma was transferred with a plastic pipette to a second ice-cold tube. An aliquot was taken for determination of the platelet concentration and platelet serotonin concentration. The platelet concentration was determined on a coulter S Plus. After centrifugation at 2000 *g* for 20 min $\frac{1}{2}$ of the volume from the central part of the plasma was transferred to a third tube (platelet-poor plasma), and stored at -20°C until assay for serotonin. Determination of serotonin was made by a radioenzymatic method as described by Hindberg (8). 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 of the assay is 0.8 nmol/l (a concentration giving rise to radioactive counts of 3 $\times$ the SD of a blank). The inter-assay relative SD is 0.11 (11%). HIOMT is specific for hydroxy indoles, and TLC in three different solvents shows identical *R_f*-values for melatonin and the radioactive product formed in the assay of serotonin.

Substance P and VIP in plasma

Ten ml blood was collected in ice-chilled test tubes containing heparin (500 IU) and trasylol (5,000 KU). After centrifugation at 1500 *g* for 10 min at 4°C, the plasma was stored at -20°C. Substance P and VIP were measured by radioimmunoassay with detection limits of 1.8 pmol/l (25) and 2.3 pmol/l (2), respectively.

Serotonin and 5-HIAA in urine

An allergen-induced liberation of serotonin from platelets into plasma may be followed by an increased turnover of serotonin. Thus, we found it relevant to measure the excretion of serotonin and 5-HIAA, the main metabolite of serotonin, in the urine. Urine was collected 4 and 20 h after nasal allergen challenge into plastic bottles containing 10 ml HCl, 6 mol/l, and stored at -20°C. Control urine samples were collected without challenge 14 days later. For 3 days before urine sampling the subjects had to omit serotonin-rich food as walnuts, bananas, tomatoes, plums, avocados and pineapples (34). No medicine was taken in the same period. 5-HIAA was determined photometrically according to the method described by Udenfriend et al. (35). The serotonin concentration was determined after dilution of 1 vol. of urine with 15 vol. of a solution of 40 g human albumin/l phosphate buffered saline (= the solution used as a blank in the assay of plasma serotonin).

Serotonin in nasal secretion

Nasal secretions were diluted with equal amounts of phosphate buffered saline containing albumin (80 g/l) added 2–3 glassbeads and shaken for 20 min to homogenize the secretions. The serotonin concentration was determined radioenzymatically with a detection limit of 1.6 nmol/l secretion. The identity of the end-product of the analysis was confirmed (as for plasma analyses) by thin layer chromatography using three different solvents (8). Further, linearity was found when serial dilutions of nasal secretions with high serotonin concentrations were analysed using the buffer for plasma analyses as diluent.

Substance P in nasal secretion

One volume of nasal secretion was extracted with three volumes of absolute ethanol. After centrifugation at 1500 *g* for 20 min the supernatant was decanted and dried in vacuum. The dried product was reconstituted to original volume with phosphate buffer before radioimmunological determination of SP (25, 30).

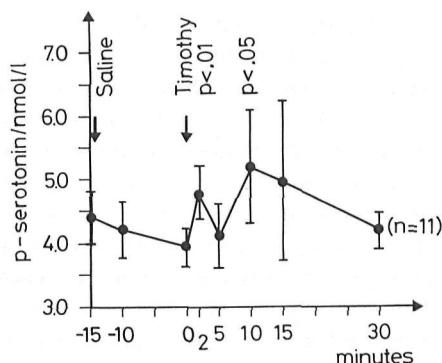


Fig. 1. Changes in plasma serotonin following nasal saline spraying and allergen provocation (mean $\pm$ SEM).

Statistics

Data were only included in the analysis when blood samples and measurement of the substance in question had been obtained at all time points. The exact number is indicated on the figures. Wilcoxon's matched-pairs test was used with a significant level of 0.05.

RESULTS

Symptom response to nasal provocation. Allergen induced itching and sneezing in all volunteers, while only two sneezed after challenge with methacholine. Allergen provocation resulted in a 30% decrease (median value) in nasal expiratory peak flow, which was significantly more ($P < 0.01$) than a 14% decrease after methacholine provocation. Allergen produced also more secretion than methacholine (1.1 vs 0.55 ml; $P < 0.01$).

Plasma and platelet serotonin. Nasal spraying with saline did not cause any change in platelet-poor plasma serotonin ("plasma serotonin") (Fig. 1). Plasma serotonin increased significantly within 2 min after allergen provocation ($P < 0.01$). Pre-challenge level was reached at 30 min. The time for the individual peak values varied among subjects.

Fig. 2 shows that platelet serotonin remained unchanged throughout the sampling period.

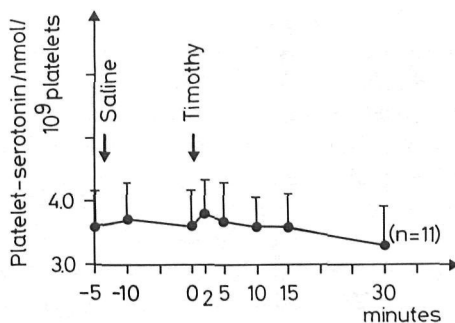


Fig. 2. Changes in platelet serotonin following nasal saline spraying and allergen provocation (mean $\pm$ SEM).

Plasma substance P. The average level fluctuated somewhat during the study period with a tendency to increase after saline spraying and decrease following allergen provocation. The scatter was considerable and no degree of significance was reached at any time (Fig. 3).

Plasma VIP. There was a rapid increase, reaching a maximum 5 min after allergen provocation and with prolonged elevation, lasting for at least 15 min ($P < 0.02$) (Fig. 4). The individual peak value for VIP was reached within 15 min, but the exact time varied among subjects.

Urinary serotonin and 5-HIAA. As seen in Table 1, there was no tendency of a change in the urine serotonin and 5-HIAA following nasal allergen provocation of 5 volunteers.

Serotonin and substance P in secretion. A measurable concentration of serotonin was found in all samples analysed, and for substance P in all, but one sample. As seen in Table 2, there was no significant difference between allergen- and methacholine-induced secretions, but the range between individual values was considerable for both substances. As allergen provocation resulted in significantly more nasal hypersecretion than methacholine provocation, the data were analysed for correlation between individual volume of secretion and concentration of mediator; neither a positive nor a negative correlation was found.

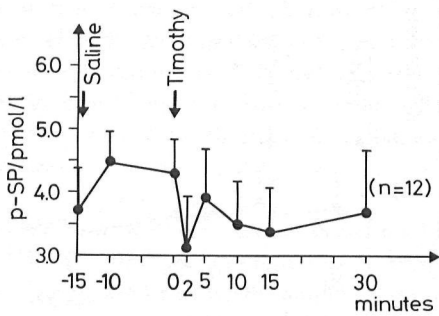


Fig. 3. Changes in plasma substance P following saline spraying and allergen provocation (mean + SEM).

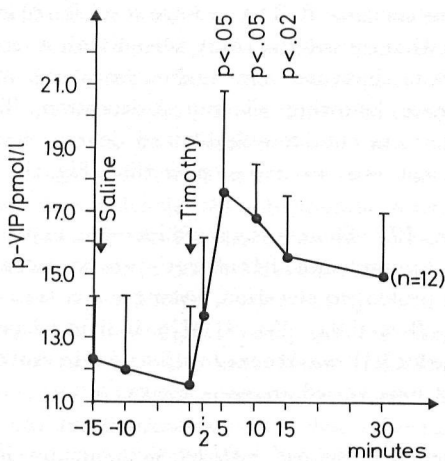


Fig. 4. Changes in plasma VIP following saline spraying and allergen provocation (mean + SEM).

The average secretion of serotonin was comparable to that of platelet-poor plasma at sampling time 5 min following allergen provocation (median values 7.2 and 4.5 nmol/l, respectively). The secretion level of substance P was very similar to that of plasma (4.0 vs 5.0 pmol/l).

DISCUSSION

In the present study we have found that allergen-induced rhinitis increases the concentration of plasma serotonin, and the increases

found in venous blood are substantially lower than in the nose. First, the platelets have an active uptake mechanism for serotonin (20), and through the passage of the lungs the endothelial cells extract about 65 % of the serotonin (3). Second, the half-life of intravenously injected serotonin is very short, about 5 min (4). Thus, moderate changes in the shock organ may not be detected in peripheral venous blood. Accidental liberation of serotonin from the platelets *in vitro*, would tend to overestimate the increase found. An important factor in determining plasma serotonin concentrations is careful handling and centrifugation of the blood samples to prevent platelet activation. That this did not occur in our study is indirectly shown by the relatively constant and low plasma serotonin concentrations in the samples during saline challenge. In accordance with this, the concentration of serotonin in plasma remained unchanged for 1 h in 3 rhinitis patients after nasal challenge with sodium chloride (unpublished observations, Hindberg, I. & Tønnesen, P.). The median increase in plasma serotonin may also be underestimated, as the individual

Table 1
Mean urinary excretion of serotonin and 5- HIAA in five pollen-allergic volunteers without and following nasal allergen provocation

		Diuresis ml	Serotonin nmol	5-HIAA µmol
Control period	0-4 h	324	186	7.3
	4-24 h	1414	1058	24.7
Allergen provocation	0-4 h	572	125	6.0
	4-24	1016	734	21.4

Table 2
Average concentrations of serotonin and substance P in nasal secretions collected over a 5 min period following a nasal provocation test; mean (range); n = 14

	Allergen	Methacholine	P-value
Serotonin (nmol/l)	7.2 (1.6-29.4)	3.7 (1.8-14.2)	NS
Substance P (pmol/l)	4.0 (0-17.5)	10.1 (4.0-15.0)	NS

peak values were reached at different times after allergen challenge. The median maximal increase – independent of time – in plasma serotonin was 31 % (range 13–342 %). Thus, in some subjects a very marked increase was observed.

The present investigation is the first attempt to relate plasma serotonin to allergy in man. Release of serotonin from platelets can be experimentally induced by platelet-activating factor (PAF) (5, 7), and PAF is liberated from basophils upon stimulation with specific antigen (6, 24). Activation of platelets in the lung during allergen-induced asthma has also been observed (9, 23). In the present study, the antigen-antibody reaction in the nose would induce release of PAF from mast cells and basophils, and PAF activation of platelets would result in release of serotonin. Thus, the most plausible source of the increase in plasma serotonin was from activated platelets in the nasal blood vessels. This is further supported by the fact that the increase and return to normal of the plasma serotonin level paralleled the changes in nasal symptoms. Because serotonin was released inside the vessels, one would expect changes in blood levels and not necessarily in nasal secretions. Nasal spraying with serotonin, although in supraphysiological dosage, induced itching, sneezing and hypersecretion, and intracutaneously injected serotonin elicited a triple response (26, 29, 31). These effects could point to serotonin as a mediator in rhinitis, although challenge experiments with physiological doses of serotonin and the effect of serotonin antagonists will be necessary to prove it.

The nasal allergen challenge was followed by a short-lasting rapid increase in the plasma concentration of VIP. The peaks were reached at different times from 2–15 min after challenge. The fluctuations in the plasma VIP concentrations were greater than the fluctuations in plasma serotonin. The source of this VIP increase remains speculative. However, VIP immunoreactive nerves have been localized in the nasal mucosa both in cat, dog and man. These nerves are located in relation to glands and vessels (32). Parasympathetic nerve stimulation

increases the VIP concentration about 500 times in the venous effluent from the nasal mucosa in the cat (13). Thus, the increase in VIP concentration found by us could be caused by release of VIP from nerves into blood vessels in the nose. As for plasma serotonin the coupling of the changes in plasma VIP and nasal symptoms also points to the source of VIP being the nose. Also VIP has effects compatible with a role in the pathogenesis of allergic rhinitis. VIP induces vasodilation of resistance and capacitance nasal vessels and stimulates glandular secretion (18, 39).

The liberation of serotonin from the platelets into the plasma, might be followed by an increased serotonin metabolism. However, no increase in the amount of serotonin or 5-HIAA was detected in the urine after allergen challenge. The elevation of serotonin in plasma occurred only for a short time, and this might be why the excretion of 5-HIAA and serotonin was not affected. In seven persons, we found the day-to-day variation in the excretion of 5-HIAA to be 8.5 % and for serotonin 12.6 %. In view of these relatively small variations, consistent changes in the excretions in our patients should have been detectable, even though the sample was small. We have found similar results for the excretion in four asthma patients with bronchial allergen provocation. In another study the amount of 5-HIAA in the urine correlated to the intensity of the symptoms in 42 asthmatics (36). In contrast, no increase in 5-HIAA in urine was found during an acute attack in eight asthmatics (38). An increase in 5-HIAA was observed in only two of 15 patients with grass pollen rhinitis in the first 2 h after allergen-provoked rhinitis (1). This is in accordance with the negative findings in the present study.

Nasal secretions in the present study contained both serotonin and SP, but their levels did not differ whether induced by allergen or methacholine. We have previously found these agents in nasal secretions in both normal subjects and rhinitis patients (27, 28, 30, 31). The ratio between the concentration of serotonin in nasal secretion and plasma (2 min after challenge) was 1.0 (median; range 0.4–5.4) and for

SP 1.0 (median; range 0.2–8.0). A higher ratio was expected for SP as its main source may be the sensory nerve endings in the nasal mucosa (30), while the main source for serotonin is most likely transudate from plasma. Because it was not possible to obtain secretions after saline nasal spraying, we could not investigate whether the concentrations of serotonin and substance P changed after allergen provocation. We attempted to overcome this problem by using nasal lavage to obtain "basal levels" (28). However, this method diluted the secretion so much that its concentration was below the assay limit in most samples. There being a lack of basal levels for serotonin, we can only conclude that serotonin and substance P were present in nasal secretions. Although this does not prove that these two agents have a role in rhinitis, it leaves the possibility open.

ACKNOWLEDGEMENTS

The authors thank J. Fahrenkrug, M.D., for the VIP analyses, and I. Jørgensen, M.Sc., for the 5-HIAA analyses.

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