

Intracutaneous and Skin Prick Testing with Serotonin and Histamine

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Skin prick and intracutaneous tests were performed with serotonin, histamine and a mixture of both in 32 normal persons, two rhinitis patients and 12 asthmatics. Skin prick tests with serotonin produced a triple response, but the weal reactions were small. Serotonin had no potentiating effect on the histamine reaction. Intracutaneously, serotonin tests also induced a triple response, but with smaller weal reactions than with histamine, and the weal surfaces were wrinkled. Constriction of the large subcutaneous veins was observed locally after serotonin injection. No quantitative or qualitative difference in reaction to serotonin was observed between normal subjects and allergics. The role of serotonin in the immediate allergic reaction in the skin was minimal.

Key words: histamine; intracutaneous test; serotonin; skin prick test.

Accepted for publication 18 November 1985

During the last 3 years we have been investigating the possible role of serotonin in the allergic inflammatory reaction in man. In contrast to earlier reports (1, 7), we found no occurrence of bronchoconstriction after serotonin bronchial challenge in asthmatics (16). However, nasal provocation with serotonin induced a rhinitis response consisting of sneezes, itching and secretion, partly atropine-sensitive (19). The secretions were partly reflex-mediated (17). Nasal discharges provoked by histamine, methacholine, and the relevant allergen, contained serotonin in concentrations many times higher than in plasma (18). Based on these findings we suggest that serotonin might be a mediator or modulator in allergic rhinitis.

Skin prick testing is another means of testing for a possible mediator and its effect in relation to well-known mediators, e.g. histamine. Prick testing with serotonin has not been previously reported, but intracutaneous (i.c.) skin tests have elicited constriction of subcutaneous veins, pain, erythema and flare, but no consistent weal reactions, except in very high concentrations (3, 8).

The aim of the present study is to further characterize the reaction to serotonin skin prick and intracutaneous testing, both quantitatively and qualitatively, and to examine whether serotonin has any potentiating effects on the histamine prick test formation.

MATERIAL AND METHODS

Subjects

The study comprised 32 normal volunteers (27 females and 5 males), two males with rhinitis, and 12 asthmatics (7 females and 5 males) with verified inhalant allergies. The mean age was 35.0 years (range 15-61). No medicine was administered at least 1 week before the test, and none of the subjects had eczema or used topical steroids.

Skin testing

Two nurses, well-trained in allergy testing, performed the prick tests by using a standard blood lancet with a point length of 4 mm and a

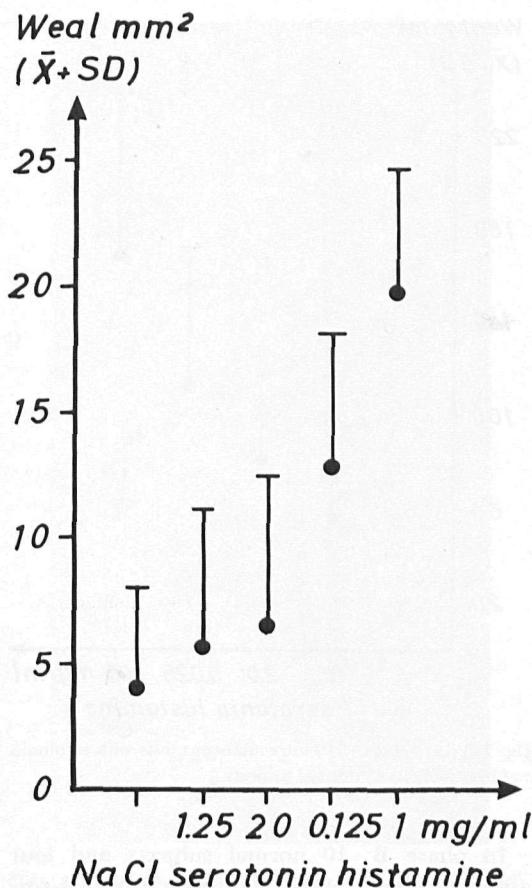


Fig. 1. Weal reactions to 96 skin pricks with serotonin and histamine in eight asthmatics.

base width of 1.8 mm. The prick tests were performed on the volar side of the forearm in one or two straight rows from 5 cm below the elbow flexure to 5 cm above the wrist. The interval between each drop was at least 2 cm. The needle was jabbed through the drop at an angle of 60–70° to the skin. The skin was penetrated with a moderate force and then lifted. After 2 min the drops were wiped off. After 10 min the weals were outlined with a fine pen and transferred by tape to square millimeter paper. The weals were measured as the greatest diameter $\times$ the diameter at right angles to its mid-point in mm². After 15 and 20 min the size of the weals was measured again. The flare formation was also measured, and any adverse reaction recorded (12). Double

controls were made in phases A and C, whereas 4-double controls were used in phase B (see Experimental design). The location of the different extracts was randomized. In phase B the extracts were coded until the statistical calculations were made. The i.c. tests in phase C and D were performed with a hypodermic needle (0.45 $\times$ 12 mm), and a volume of 0.05 or 0.02 ml was injected i.c. (22).

Reagents

The reagents used were serotonin creatinine sulphate (serotonin) 0.5, 1.25, 2.0, 2.5, 5.0, 10.0, and 20.0 mg/ml in a phosphate buffer (pH 7.4), and histamine chloride (histamine) 0.0125, 0.025, 0.05, 0.1, 0.125, 0.5, 1.0, and 10.0 mg/ml, also in phosphate buffer (pH 7.4). The following mixtures of serotonin and histamine were used in phase B: serotonin 10 mg/ml + histamine 1 mg/ml; serotonin 20 mg/ml + histamine 1 mg/ml; serotonin 10 mg/ml + histamine 10 mg/ml; serotonin 20 mg/ml + histamine 10 mg/ml. All reagents were supplied by the Central Hospital Pharmacy, Herlev, Copenhagen and stored at 4°C for not more than 72 h.

Experimental design

The study consisted of four parts. In phase A skin prick tests were performed in eight asthmatics using different concentrations of serotonin and histamine in an open trial. Phase B was carried out as a double-blind experiment with prick tests in 14 persons (4 asthmatics) with serotonin, histamine, and serotonin and histamine combined, to examine any possible interaction in effect between the two reagents. In phases C and D i.c. tests were done with serotonin and histamine in an open study in 22 normal subjects and two rhinitis patients to study the weal and flare reaction quantitatively as well as qualitatively.

Statistics

Wilcoxon's test for paired data was used to test difference in response to histamine and

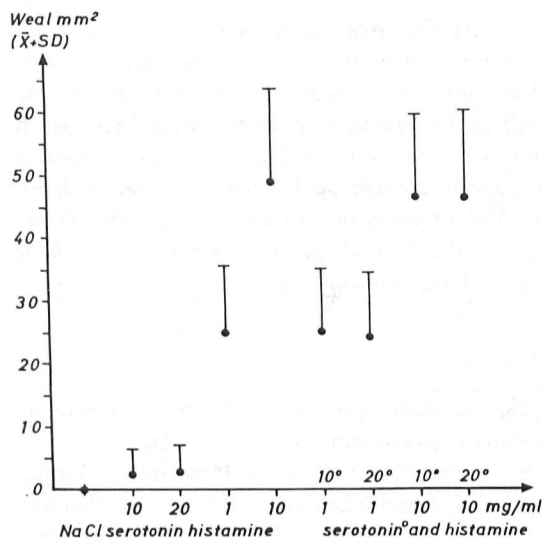


Fig. 2. Weal reactions to 504 skin pricks with serotonin, histamine and serotonin + histamine in 14 subjects (4 asthmatics).

serotonin. Mann-Whitney's test for unpaired data was used to calculate any difference between normal subjects and asthmatics.

RESULTS

In phase A, eight asthmatics were tested with a total of 96 pricks (Fig. 1). Histamine and serotonin induced a triple response in the skin, consisting of local flare, erythema and weal formation. The weal reaction after serotonin was small, but greater in size than in the negative control with isotonic saline ($P < 0.01$). Many of the weals had a diameter < 3 mm, which is normally classified as a negative unspecific reaction to local trauma. There was no significant difference between the weal area after serotonin 1.25 mg and 20.0 mg/ml ($P > 0.1$), in contrast to a dose-dependent increase in weal size to histamine 0.125 and 1.0 mg/ml ($P < 0.01$). The flare reaction had a mean diameter of 25 mm to histamine 1.0 mg/ml and 23 mm to serotonin 20 mg/ml ($P > 0.1$). Both the weal and flare formation after serotonin was maximal in size after 8–10 min, and no increase was observed after 15–20 min. No late reactions (24 h) were reported. The serotonin flare was more intensely red, and more indistinctly margined in contrast to the histamine flare.

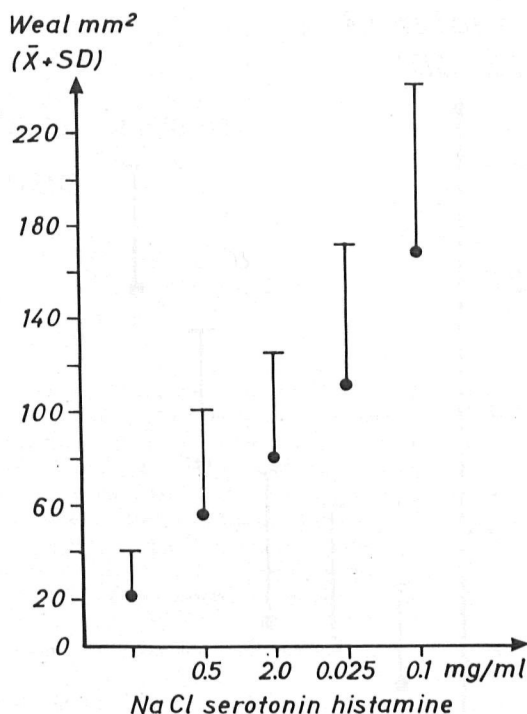


Fig. 3. Weal areas in 140 intracutaneous tests with serotonin and histamine in 14 normal subjects.

In phase B, 10 normal subjects and four asthmatics were tested with a total of 504 pricks. From Fig. 2 it appears that the weal reactions after serotonin (10 and 20 mg/ml) were small, but again statistically different from the inactive control ($P < 0.01$). Histamine (1 and 10 mg/ml) produced a dose-dependent increase in reactions ($P < 0.01$). Serotonin had no additive or potentiating effect on the response to histamine ($P > 0.1$). The standard deviation in percent for the difference in weal area between 4-double-controls was 21.4–39.1% for serotonin, histamine and histamine and serotonin combined. The reaction to serotonin 20 mg/ml in the 10 normal subjects was not statistically different from the reactions in the 12 asthmatics in phases A and B, either quantitatively or qualitatively. The weal area in the normal subjects was 3.8 ± 3.7 and 4.2 ± 5.5 in the asthmatics ($P > 0.1$). The asthmatics have therefore been included in the calculations for the normal subjects.

In phase C, 140 i.c. tests (0.05 ml) were

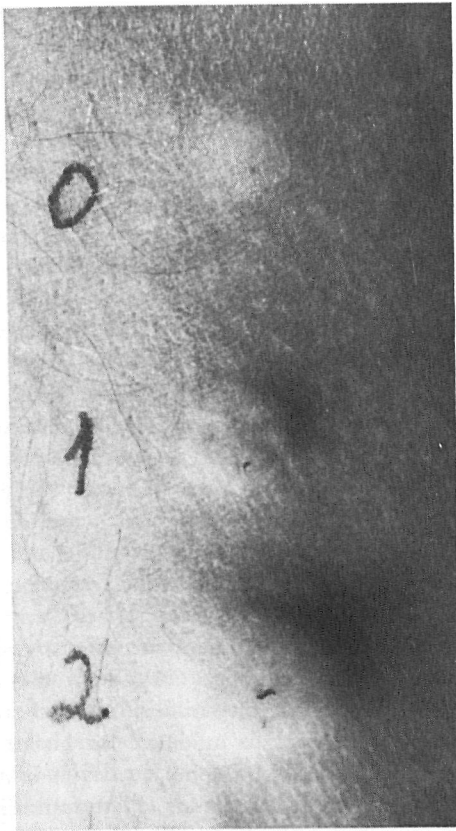


Fig. 4. Intracutaneous tests with isotonic saline (0), serotonin creatinine (2.5 mg/ml) (1) and histamine chloride (50 µg/ml) (2) on the volar site of the right forearm in a normal male student, ($\times 1.2$).

performed in 14 normal volunteers. From Fig. 3 it is apparent that the weal reaction to both histamine (1.25 and 5 µg) and serotonin (25 and 100 µg) showed a dose-related increase ($P < 0.01$). The time course was almost similar for both agents, with maximal reactions after 10 min. No late reactions were observed. Histamine was 100 to 400 times more potent than serotonin in the ability to induce weals. No statistically significant dose-response curve was found for serotonin and histamine. With histamine the weal surface was smooth, while with serotonin the surface appeared wrinkled with many pin-point retractions resembling a piloerection (Fig. 4). The weals were not affected by whether the test was performed on the left or right arm ($P > 0.1$). There was a linear correlation between the individual response to histamine 1.25 µg and serotonin 25 µg ($r = 0.91$, $P < 0.01$) and between histamine 5 µg and serotonin 100 µg ($r = 0.61$, $P < 0.05$). The standard deviation in percent for the difference in weal area between double-controls was 15.6–19.3 % with serotonin and 36.9–43.6 % with histamine.

In phase D, 50 i.c. tests were done in eight normal subjects and two rhinitis patients with serotonin (1 µg, 25 µg, 100 µg) and histamine (0.25 µg, 1 µg) separately on the left and right forearm randomly. Table 1 shows that serotonin induced considerable venoconstriction of the large subcutaneous veins in all 10 persons.

Table 1

Response to intracutaneous tests with serotonin and histamine on the volar site of left and right forearms in eight normal subjects and two rhinitis patients. Injected volume was 0.02 ml. Mean values $\pm$ SD. Range in parenthesis

	Serotonin			Histamine	
	1 µg	25 µg	100 µg	0.25 µg	1 µg
Venoconstriction		87.4 $\pm$ 51.9 cm ² (35–200)			0
Erythema (cm ²)	5.4 $\pm$ 2.7 (1–12)	7.7 $\pm$ 2.5 (5–12)	10.3 $\pm$ 5.2 (6–20)	5.4 $\pm$ 3.1 (1–12)	6.8 $\pm$ 3.4 (4–12)
Weal area (mm ²)	32.1 $\pm$ 15.0 (14–56)	60.2 $\pm$ 23.1 (30–96)	86.6 $\pm$ 27.3 (51–135)	73.0 $\pm$ 30.2 (12–114)	112.1 $\pm$ 49.3 (70–240)
Weal duration (min)		134 $\pm$ 51 (60–200)			108 $\pm$ 31 (70–145)
Pain		2 severe/4 moderate			0/10
Itching		6/10			5/10

The vasoconstriction involved a large area of the forearm, and in two persons the whole forearm was involved and the veins of the hand engorged; they experienced a moderate to severe pain in the forearm and elbow flexure. The serotonin-induced erythema was again a more intense red, and after 5–10 min small red spots appeared up to 5 cm from the border. Erythema and vasoconstriction were also induced by 1 µg serotonin. The duration of the weal reaction tended to last longer after serotonin ($P < 0.1$). There was no quantitative or qualitative difference in the response to serotonin between the two rhinitis patients and the healthy subjects.

DISCUSSION

Skin prick testing with serotonin in the highest possible concentration soluble in water (20 mg/ml) produced only insignificant weal reactions, but the erythema and flare reaction was found in most subjects after both serotonin and histamine. With prick testing, serotonin showed no potentiating or additive effect on the histamine response, although a small additive effect might have been missed because the histamine concentration used was too high in relation to that of serotonin, but does not seem likely for the low concentration of histamine 1 mg/ml.

With i.c. administration, serotonin induced a triple response with erythema followed by a flare and weal reaction with a dose-dependent increase in weal size. In our study 1 µg and 25 µg serotonin (0.5 mg/ml) produced a weal in all subjects in agreement with an earlier report, where the same dose induced weals in the majority of the subjects (3), while smaller doses of serotonin did not (2, 8, 13). We, in accordance with others (24), found no exaggerated response to serotonin in asthmatics, in contrast to patients with rheumatoid arthritis. These patients showed an exaggerated response with cyanosis of the fingers to serotonin 0.1 mg i.c. in the fingers (10, 15).

Demis et al. (3) reported that the flare reaction to serotonin could be inhibited by local anesthetics, but not when used in subanesthetic

concentrations. Vasoconstrictors (epinephrine, norepinephrine, ergotamine tartrate) were also effective inhibitors of the flare formation. These results support the general view that the flare reaction is mediated by axon reflex vasodilation. The difference in the flare reaction between serotonin and histamine, with a more mottled and indistinct margin with serotonin, can be explained by action at different arteriolar levels and/or varying degree of dilation of the same vessels. A purely quantitative effect on the vessels seems the most plausible.

Most reports find that serotonin does not increase capillary permeability in concentrations up to 0.1 mg/ml; but in higher concentrations serotonin can increase capillary exudation, as measured by extravasation of Evans Blue (3, 11, 14). Anti-histamine, but not anti-serotonin drugs could reduce the exudation, suggesting that the effect of serotonin on capillary permeability (weal formation) might be mediated by histamine (11). Serotonin has been able to release histamine in animal trials (5). Serotonin had no influence on histamine release from human basophil leucocytes *in vitro* (Stahl Skov, P. & Tønnesen, P.: unpublished observations). Thus the histamine releasing effect of serotonin in man remains obscure. Theoretically, serotonin might also stimulate the sensory fibres in the epidermis, which could initiate an orthodromic impulse to cause release of Substance P (SP) causing vasodilation and plasma extravasation (the axon reflex) (9). This stimulus might also release histamine from mast cells, and together induce the weal and flare formation. SP has been shown to release histamine from rat mast cells without interacting with cell-bound IgE. Intradermal injection of SP into human skin produces weal and flare reactions (6). The flare formation is sensitive to H_1 receptor antagonists and to compound 48/80. Thus, some of the vascular changes of SP are indirectly brought about by the release of histamine in the skin. In the nose we found that serotonin challenge induced nasal secretions which contained SP in concentrations parallel to increasing provocative doses of serotonin (20).

In the present study serotonin also elicited constriction of the large subcutaneous veins,

first described by Reid in 1952 (13). This effect of serotonin is unique, and might be a result of increased sympathetic activity, also indicated by the piloerector reaction described above. However, a direct effect of serotonin on the veins is a more plausible possibility. We found no signs of nasal venoconstriction after serotonin application, measured indirectly by nasal airway resistance (19). However, it is well known that serotonin can induce vasoconstriction or dilation, depending on the anatomical origin of the vessel, the species, the concentration of serotonin, the background sympathetic activity, and the state of the endothelium (4). Also 1 μ g serotonin produced venoconstriction, flare and weal reactions. The serotonin content of human skin is below 0.01 mg/g tissue (21, 23), thus the serotonin concentration used (1 μ g) is in the physiological interval. Venoconstriction was not observed in response to histamine, and is not observed after allergen skin testing. The weal reaction after histamine and allergen appears with a smooth surface in contrast to the wrinkled surface observed after serotonin i.c. injection. Thus, the role of serotonin in the allergic immediate skin reactions appears to be minimal in man. That serotonin did not potentiate histamine skin tests supports this statement. Histamine was also 100–400 times more potent than serotonin in its ability to induce weals, although a linear dose-response curve was not documented. With regard to erythema formation, serotonin was equipotent to histamine, but with serotonin the erythema was more intense. Thus, serotonin might play a role in the flare formation in the skin.

In summary, skin testing with serotonin produced a triple response and venoconstrictions. The weal reaction is produced by high and unphysiological concentrations. No difference was found between normal subjects and allergies in their reaction to serotonin.

ACKNOWLEDGEMENTS

Nurses Hanne Steensgård and Lone Pedersen are thanked for their skilled assistance during the study.

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