

Cutaneous and basophilic sensitivity to substance P and gastrin in non-atopic versus atopic subjects

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We compared the cutaneous reaction to intradermal injection of substance P, gastrin and histamine in asymptomatic atopic subjects with a history of hay fever and/or asthma versus non-atopic healthy volunteers. We also studied in these two groups the basophilic histamine release induced by substance P and gastrin with that obtained with anti-human IgE and Con A. Intradermal injection of substance P (3-300 pM) and gastrin (3-30 pM) caused a wheal and flare reaction which was comparable in both groups of subjects. Substance P 10^{-4} M caused a mean basophilic histamine release of about 15% in atopic and non-atopic subjects. Gastrin was not effective in this model. Anti-IgE and Con A-induced histamine release was significantly higher in atopic than in non-atopic volunteers.

Key words: atopics; peptides; sensitivity, cutaneous, basophilic.

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Substance P is a sensory neuropeptide which has been recently implicated in the pathogenesis of bronchial asthma as a possible neuromediator in axon reflexes (2). Intradermal injection of substance P in man causes a wheal and flare reaction which is, at least partly, due to local histamine release (11, 14). Substance P at high concentration induces *in vitro* histamine release from human skin mast cells (3, 17). We also found that substance P (10^{-4} M) induces a modest histamine release from human lung mast cells and basophils (16).

It has been reported that the hormonal polypeptide gastrin could induce histamine release from infant foreskin *in vitro* and a wheal and flare reaction when intradermally injected in man (25). The effect of gastrin on human basophil has never been studied. Some data in the literature suggest that basophils and skin mast cells from atopic patients release their mediators more readily than normal individuals when triggered *in vivo* or *in vitro* (4, 5, 12,

15, 19, 21). We therefore considered it interesting to compare in these two groups cutaneous and basophilic sensitivity to substance P and gastrin, which are two peptides with a possible role in human mast cell and basophil secretion.

SUBJECTS AND METHODS

Fourteen non-atopic healthy subjects and 14 atopic patients volunteered for this study. Both groups were matched by age and sex (age range: 25-55 years). Atopic subjects were hay fever or asthmatic patients who were asymptomatic at the time of the study. They were atopic by history and had positive skin tests and RAST for grass pollen, house dust and *Dermatophagoides pteronyssinus*. Non-atopic subjects had negative history, skin tests and RAST for a battery of antigens. None of the subjects was taking medicines for 3 weeks prior to skin test and blood sampling.

Intradermal injection

Fresh solutions containing histamine diphosphate (Sigma, St Louis, MO), substance P or gastrin (UCB bioproducts, Braine-l'Alleud, Belgium) in appropriate concentrations were separately injected intradermally (0.03 ml) with a 1-ml tuberculin syringe in the forearm of the volunteers. Skin reactions were evaluated 15 min later by measuring two perpendicular diameters of the wheal and flare reactions, including the longest axis and ignoring small pseudopodia. Results were expressed as the mean of the two perpendicular diameters. Only wheal and flare reactions > 5 and 8 mm respectively were considered as positive. Fresh solutions of histamine (1 $\mu\text{g/ml}$), substance P (10^{-9} , 10^{-7} and 10^{-5}M) and of gastrin (10^{-9} , 10^{-7} and 10^{-6}M) were made in saline Hepes the day of the injection.

Leukocytic histamine release

Venous blood (60 ml) was drawn into vacutainers (Becton Dickinson) each containing lithium heparin (143 USP units/tube) and 1 ml plasma-gel (Bellon, Neuilly, France). The blood was

gently mixed and allowed to sediment for 30 to 60 min at 37°C . The upper plasma leukocyte layer was removed from each tube, pooled and centrifuged at 350 g for 8 min. The supernatant plasma was decanted. The packed cells were washed twice in 15 ml of cold Hanks' without Ca^{++} , Mg^{++} and phenol red (Gibco, Scotland). Finally, the cells were resuspended in Hanks' containing Ca^{++} and Mg^{++} (Hanks' -CM) to approx. 10^7 leukocytes/ml. To 1 ml of this leukocyte suspension was added the same volume of a solution containing substance P or gastrin, in appropriate concentration, and Con-A (Sigma, St. Louis, MO) or antibodies to human IgE (Behring, Marburg, Germany) in optimal concentrations. The mixture was incubated for 30 min at 37°C . Cells and fluid were separated by centrifugation. The fluid phase was deproteinized by addition of the same volume of $\text{HCl}_{0.4}$ (0.8 N) and the supernatant obtained after centrifugation analysed for histamine content. Blanks consisted of deproteinized supernatant of leukocytes incubated with Hanks' CM. For determination of total histamine, 1 ml duplicates of leukocyte suspension (10^7 cells) were treated with the same volume of 0.8 N $\text{HCl}_{0.4}$ and centrifuged, and the supernatant was assayed for histamine. Histamine was measured by an automated spectrofluorometric assay. Secretagogue-induced histamine release was expressed in per cent of the total histamine of the leukocyte suspension.

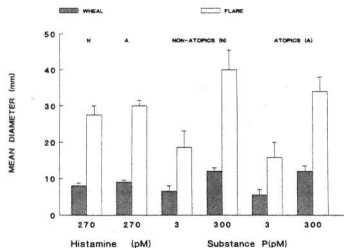


Fig. 1. Comparison of skin wheal (hatched bars) and flare (plain bars) to histamine and substance P in atopic and non-atopic subjects. Responses were expressed as the mean diameter of two perpendicular diameters including the longest axis.

Statistical analysis

The data were analysed using the Student's impaired t test. A P value < 0.05 was considered significant. All values are presented as mean ($\pm$ SEM).

RESULTS

Skin reaction

Fig. 1 shows the mean ($\pm$ SEM) wheal and flare reactions observed in 14 non-atopic volunteers and in 14 atopic subjects following intradermal

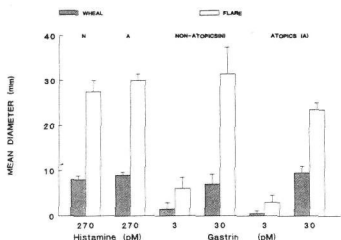


Fig. 2. Comparison of skin wheal (hatched bars) and flare (plain bars) to histamine and gastrin in atopic and non-atopic subjects. Responses were expressed as the mean diameter of two perpendicular diameters including the longest axis.

injection of histamine (270 pM) or substance P (3 and 300 pM). Intradermal injection of substance P 0.3 pM caused no cutaneous reactions. At 3 pM, it induced a reaction in about 60% of the subjects in both groups and, finally, at the dose of 300 pM, it elicited a wheal and flare reaction in all the subjects of both groups. On a molar basis, the wheal reaction induced by substance P was significantly higher than that induced by histamine in both groups ($P < 0.05$).

Mean values of the wheal and flare reaction observed with gastrin in normal and atopic subjects are shown in Fig. 2. Gastrin 0.3 pM failed to induce any reaction. However, at 3 pM, a wheal and flare reaction was observed in about 20% of subjects in both groups and, finally, at 30 pM, gastrin caused in all the subjects a significant response of the same magnitude in normal and atopic subjects. On a molar basis, gastrin was more effective than histamine in inducing a wheal and flare reaction when injected intradermally in man.

Leukocytic histamine release

Substance P (10^{-4} M), but not gastrin, caused a histamine release from human basophils. The mean histamine release induced by substance P (10^{-4} M) was 16% and 13% respectively in non-

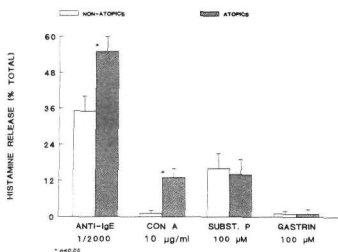


Fig. 3. Comparison of basophilic histamine release induced by anti-IgE, ConA, substance P and gastrin in non-atopic (plain bars) and atopic subjects (hatched bars). (* significant difference at $P < 0.05$).

atopic healthy volunteers and in atopic subjects. This difference is not significant ($P > 0.05$). Substance P-induced basophilic histamine release was significantly weaker than that achieved by optimal dose of anti-human IgE. In contrast to substance P, ConA and anti-IgE caused a significantly higher histamine release in atopic compared with non-atopic subjects ($P < 0.05$) (Fig. 3).

DISCUSSION

This work confirms previous studies showing that intradermal injection of substance P or gastrin causes a wheal and flare reaction in man (11, 14, 25). We show here that the cutaneous reactivity to intradermally injected substance P or gastrin (or histamine) of asymptomatic atopic subjects is similar to that observed in non-atopic healthy volunteers. Indirect evidence suggests that the cutaneous reaction to intradermal injection of substance P is, at least partly, due to a local histamine release. Indeed, the flare component can be prevented by pretreatment with a histamine H_1 antagonist or with a mast cell stabilizer, doxantrazol (14). Moreover, substance P causes histamine release from human skin mast cells *in vitro* (3, 17). However, other mediators might be implicated in cutaneous reaction to substance P since this one can also

stimulate prostaglandin and leukotriene secretion from skin mast cells (6). Increased vascular permeability induced by substance P may also lead to kinin formation. In contrast to substance P, there is no convincing argument to suggest that the wheal and flare reaction induced by gastrin is due to a histamine release. Tharp et al. found that gastrin induced a histamine release from infant foreskin *in vitro* and that wheal and flare reaction due to gastrin was enhanced in patients with mastocytosis (25). However, results obtained by Benyon et al. (3) and also by us (unpublished data) using human skin mast cells did not confirm any effect of gastrin on histamine release. Further, this study shows that gastrin is unable to stimulate histamine release from human basophils. Contrary to gastrin, substance P at high concentration does induce a significant histamine release from basophils in atopic and non-atopic subjects. It has been demonstrated that substance P at micromolar concentrations induces a histamine release from rat serosal mast cells (9, 13, 23). We found that substance P in the same range of concentration could also induce histamine release from human lung mast cells and basophils (16). The amount of histamine released is, however, modest and always smaller than that achieved by an optimal concentration of anti-IgE. Our results show that substance P-induced histamine release, contrary to those induced by anti-IgE or ConA, has the same magnitude in atopic as in non-atopic subjects. It is generally admitted that IgE-dependent secretagogues, such as anti-IgE, ConA or protein A, cause a greater basophilic histamine release in subjects suffering from atopic disorders (4, 19) and/or asthma (1, 10).

However, the mechanism of the high basophil releasability of atopics and asthmatics remains poorly understood. The sole fact that atopics present a higher IgE density on the surface of their basophils is not sufficient to explain the high histamine release in these patients (7). One can attempt to explain this phenomenon in qualitative rather than quantitative terms as far as membrane-bound IgE molecules are concerned. Indeed, cell-bound IgE could be different in their capacity to bridge with secretagogues (15). What is more certain IgE, referred

as to IgE+ and associated with a state of atopy, are capable of rendering basophils more sensitive to a number of non-immunologic stimuli, such as HRF and IL3 (20, 21). It has also been observed that basophils from atopic subjects undergo a higher histamine release when activated by PAF compared with basophils from normal individuals (22). Experiments using formylmethionine-containing peptides (FMP) failed to demonstrate a greater activation of basophils in atopic patients compared with normal individuals (8).

FMP causes histamine release from human basophils by interacting with specific receptors (24). Substance P is an undecapeptide bearing a partial resemblance in aminoacid sequence with formylmethionine leucine phenylalanine. It has been shown capable of interacting with the FMP rabbit neutrophil receptors (18). Our finding of a similar releasability to substance P in atopic subjects is consistent with the concept that substance P is an IgE-independent secretagogue interacting with peptide receptors on the human basophils (which are probably shared by other oligopeptides).

In conclusion, intradermal injection of substance P and gastrin in man causes a wheal and flare reaction which is comparable in asymptomatic atopic versus non-atopic healthy volunteers. Substance P, but not gastrin, causes histamine release from human leucocytes. In contrast to anti-IgE or ConA, substance P-induced basophilic histamine release has the same magnitude in atopic as in non-atopic subjects, indicating a mode of action differing that of IgE-dependent secretagogues.

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