

ORIGINAL ARTICLE

Re-sensitization of desensitized histamine H1 receptors in the human skin takes more than 18 hours

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Funding information

Kungliga Fysiografiska Sällskapet i Lund, Grant/Award Number: 31905

Abstract

Background: It is known since the time of testing histamine on pieces of guinea pig's jejunum that histamine receptors can develop insensitivity. The aim was to find evidence for desensitization of histamine H1 receptors in the human skin in vivo and, if found, to study the time for such receptors to regain normal sensitivity.

Materials and Methods: A skin prick test with histamine (10 mg mL⁻¹) was set in areas where a large histamine wheal was evoked 2, 6, 18, 24 or 72 hours earlier. A skin prick test with histamine (10 mg mL⁻¹) was also set in an area where an allergen wheal was evoked 2 or 6 hours earlier. Heights, diameters and areas were measured on photographs of side views of plaster casts of the evoked skin elevations.

Results: Histamine wheals, called test wheals, in areas where large histamine wheals were evoked 2, 6 or 18 hours earlier, were smaller than histamine wheals, called initial wheals, in earlier non-stimulated areas. Test wheals from the 18 hours experiment were smaller than test wheals from the 72 hours experiment. Test wheals evoked in areas where allergen wheals were evoked 2 or 6 hours earlier were smaller than corresponding initial wheals.

Conclusion: Histamine-evoked wheals and IgE-mediated allergic wheals reduce the sensitivity of histamine H1 receptors in the human skin. It takes between 18 and 72 hours to restore the sensitivity. Similarities between the development of histamine wheals in the human skin and histaminergic migraine with aura are discussed.

KEYWORDS

desensitization, histamine H1 receptors, human skin wheals, migraine with aura, re-sensitization

1 | INTRODUCTION

In a study of medical students who were skin prick tested with histamine, we detected a colour shift of the wheals from pale red to darker red between the 18th and the 20th minutes after the skin prick test (SPT).¹ One suggestion to explain the colour shift found was total desensitization of histamine H1 receptors (H1 receptors) in turn causing change of post-capillary resistance vessels from vasoconstriction to vasodilatation.

The aim with the present study was to find evidence for such a desensitization and, if found, to study the time necessary for re-sensitization. To investigate that, magnitudes of histamine wheals were compared. Histamine wheals evoked in previous non-stimulated human skin areas were compared to histamine wheals evoked in skin areas where histamine wheals were evoked different hours earlier. To measure diameters of wheals directly on the skin was not accurate enough for the present aims. Instead, a technique shortly described in the above article was used.¹ Casts were taken of histamine

wheals, first negative ones with a fast stiffening material and from them positive ones with plaster of Paris. Measurements were then done on a computer screen of magnified photographs taken of the positive casts. The technique of the casts is here evaluated more thoroughly, and the advantages described. Histamine wheals were also evoked in previous allergen wheals in order to see if mast cell release was able to reduce the sensitivity of histamine receptors.

2 | MATERIALS AND METHODS

2.1 | Subjects

1. Six consecutively enrolled healthy males aged 39-68 (mean 50 years) were tested with histamine into skin areas where they 2, 6, 18, 24, or 72 hours previously had a large histamine wheal.
2. Twelve consecutively enrolled patients with allergic rhinitis, 6 males and 6 females, aged 26-55 (mean 38 years), were tested outside their allergy seasons with histamine into skin areas where previously allergen wheals were evoked. Six of the patients were tested after 2 hours and the other six ones after 6 hours.

2.2 | The healthy subjects with large histamine wheals

Histamine skin prick tests (SPTs) were set with histamine dihydrochloride solution, 10 mg mL⁻¹, (Soluprick®, ALK-Abelló A/S). The skin of the volar side of the forearm was penetrated through a drop of the solution with a lancet (ALK Lancet®, ALK-Abelló A/S). The lancet was held in place for 3-4 seconds. The drop was wiped away after 1 minute. The whole procedure was performed according to the routine of the allergy office at our ENT department. A wheal evoked in an earlier non-stimulated area is in the following called initial histamine wheal or initial wheal.

Histamine wheals evoked with such a technique reach their maximal size after around 18 minutes.¹ Exactly 15 minutes after the histamine SPT was set a negative cast of the evoked initial wheal and the surrounding skin was taken. Then, in order to get a wheal larger than expected histamine wheals and of around the same size as allergen wheals, four histamine SPTs were set in the borders of the still existing initial wheal. Fifteen minutes later a negative cast was taken of the evoked large histamine wheal. Within that area where large histamine wheals were evoked 2, 6, 18, 24 or 72 hours earlier one histamine SPT was set. The wheal evoked by such a histamine SPT, used to test the sensitivity, are in the following called test histamine wheal or test wheal. Some minutes before the histamine SPT for the test wheal was set another negative cast was taken, in order to observe if a skin elevation of the large histamine wheal still existed at that time. The whole procedure is described in Figure 1.

The negative casts consisted of ALGINoplast® (Heraeus Kultzer GmbH), a fast stiffening material when stirred in water. ALGINoplast is used by dentists for teeth impressions. In order to get a lasting

imitation of a wheal a plaster of Paris cast, below named positive cast, was performed from the negative cast around half an hour later. The same circular form with a diameter of 3.5 cm was used for both the negative cast and the positive one. The positive plaster cast of the test wheal was then compared to the positive cast of the initial wheal regarding the photographed side view of diameter, height and area.

Each subject completed five series of histamine SPTs evoking initial wheals, large wheals and test wheals. Between each series for the same subject there were several weeks. None of the subjects were tested at the same days. All histamine SPTs were performed and all casts, negative ones and positive ones, were taken by the author.

2.3 | The patients with allergen wheals

The patients, all of them earlier diagnosed with SPTs, came to the allergy office where a nurse performed different allergen SPTs (Aquagen®, ALK-Abelló A/S) and one histamine SPT (Histamine dihydrochloride solution, 10 mg mL⁻¹, Soluprick®) on the volar side of the forearm as described for the healthy subjects. A negative cast was taken exactly 15 minutes after the histamine SPT was set and another negative cast of the largest allergen wheal around 17 minutes after the allergen SPTs were set. Two, respectively, 6 hours later the patients of the two allergen groups were tested with a histamine SPT at the site of the selected allergen wheal. Just before the histamine SPT for the test histamine wheal was set a negative cast was taken, in order to observe if a skin elevation of the allergen wheal still existed.

2.4 | Measurements and calculations

Photographs were taken as side views in six different angles of each of the positive casts. The six photographs were then handled in an iMac, OS X and magnified. Positive casts of wheals are in the following called wheals. Arbitrary values of a wheal were obtained with ImageJ, a calculation program from NIH. At first the area of each

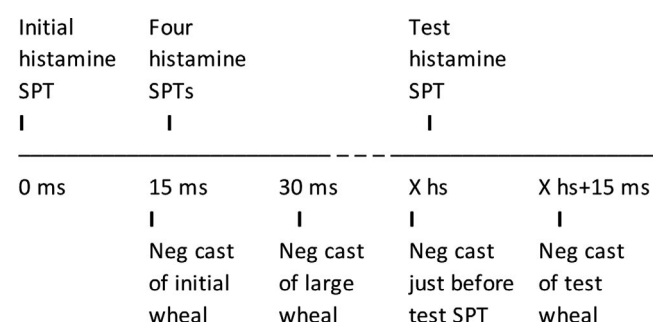


FIGURE 1 Flow chart for the healthy subjects showing when initial histamine skin prick test (SPT) and test histamine SPT are performed and when negative (Neg) casts of histamine wheals are taken. X hs stands for 2, 6, 18, 24 or 72 h and ms is here short for min

of the six photographs was measured six times. The photograph of the wheal with the area with highest mean value was then selected for measuring arbitrary values of diameter, height and area. Such a selected photograph is seen in Figure 2 with description of how the diameter, the height and the area are defined. Heights and diameters in mm were also obtained using a digital calliper or a ruler directly on the screen and a millimetre scale on the photographs. The calliper had a window for figures in mm with two decimals. The ruler was read with an accuracy of 1 mm.

A person, who had no knowledge of the aims of the study, arranged all the selected photographs from the 18, 24 and the 72 hours experiments in a blinded way. The photographs of all wheals of the 2 hours and the 6 hours experiments, both from the healthy subjects and from the patients, were not arranged in a blinded way. The reason was that almost all those test wheals had no distinct borders, and consequently, no diameters could be measured. As the height and area of each test wheal were intended to be compared to those of the corresponding initial wheal a diameter was needed. It was decided that as diameter of a test wheal with no borders the length of the diameter of the corresponding initial wheal was used.

Height, diameter and area were each measured 6 times and mean value and standard deviation (SD) of mean value were calculated. The SDs of the mean values of all the arbitrary values were usually less than three per cent of the mean values (coefficient of variation). The measures obtained by the calliper or the ruler on the screen were calculated with Microsoft Excel (version 14.0.0). Their SDs of the mean values were also usually less than 3% of the mean values. There was also a possibility to measure directly on the positive casts with the calliper. However, with that technique heights of wheals from subjects and patients usually had SD that was around 30% of the mean value. Therefore, results with that technique are not presented except diameters of the allergen wheals and the large histamine wheals. Those wheals were not circular. The longest and shortest diameter of each wheal were measured with the calliper directly on the positive cast and the mean value calculated.



FIGURE 2 The photograph represents an initial histamine wheal of one of the healthy subjects. The definition of diameter here is the distance between the two borders where the initial wheal meets the surrounding skin surface. Height is the distance of a line perpendicularly to the diameter and to the highest point of the wheal. The area is all of the wheal above the diameter

The author took photographs of all the positive casts and made all the measurements and calculations. A number of photographs, ie, those from the 18 hours test histamine wheals and the 72 hours test histamine wheals, were also taken by a person, who had no knowledge of the aim of the study. The intention with those latter photographs was to control if the measurements and the calculations gave similar results as when the photographs were taken by the author.

2.5 | Statistics

The results are given in mm as mean values $\pm$ SD of means. Data were analysed by using paired Student's *t*-test, two ways. *P* values of less than .05 were considered significant.

2.6 | Ethical permission

Ethical approval was obtained by the regional Ethical Committee at Lund University Hospital, Sweden (Dnr 2013/5). All patients and healthy subjects gave written consent prior to participate in the study.

3 | RESULTS

3.1 | The healthy subjects with large histamine wheals

The large histamine wheals were not circular. The mean diameter and SD for the large histamine wheals of the six subjects in the 2 hours experiment was 11 ± 1 mm and for the same six subjects in the 6 hours experiment 11 ± 2 mm. The mean diameters of the large histamine wheals of the 18 hours experiment, the 24 hours experiment, or the 72 hours experiment were also around 11 mm.

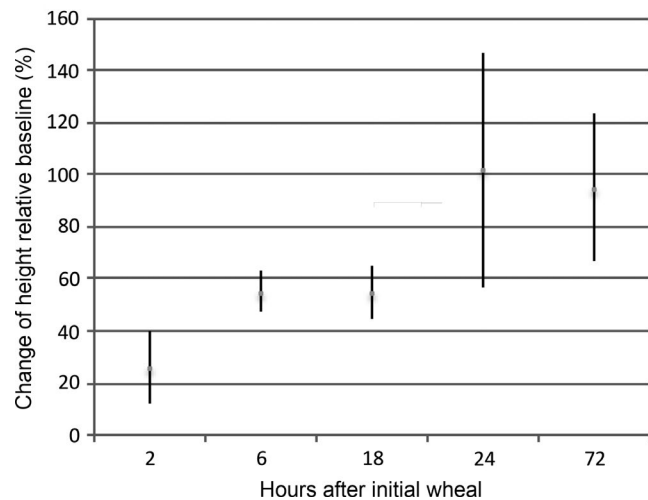
All initial histamine wheals were circular and had distinct borders. All test wheals from the 18, 24 or 72 hours experiment also had distinct borders. To judge from the photographs, the test histamine wheals from two of the subjects of the 6 hours experiment had uncertain borders. However, when the casts were observed directly by the naked eye none of them had wheals with distinct borders all around.

The heights of the 2, 6 or 18 hours test wheals were lower than the heights of corresponding initial wheals. The results in mm are given in Table 1. The heights in mm of the 2 hours test wheals were lower than those of the 6 hours test wheals ($P = .014$). The heights of the 2 hours test wheals or the 6 hours test wheals were both lower than those of the 24 hours test wheals or the 72 hours test wheals (all *P* values $< .034$). The heights of the 18 hours test wheals were lower than those of the 72 hours test wheals ($P = .043$) but not lower than the 24 hours test wheals ($P = .059$). Mean heights $\pm$ SDs of test wheals in per cent of baseline (heights of initial wheals) calculated from the mm-values are shown in Figure 3. The heights were usually 5%-10% of the diameters of

TABLE 1 Heights of histamine wheals in previous large histamine wheals

	Initial wheals	Test wheals	Difference
2 h	.40 ± .09	.10 ± .05	$P = .001$
6 h	.44 ± .14	.26 ± .10	$P = .001$
18 h	.49 ± .10	.27 ± .04	$P = .003$
24 h	.43 ± .10	.42 ± .15	n.s.
72 h	.39 ± .07	.38 ± .11	n.s.

Note: Mean heights ± SD of means in mm of initial histamine wheals in previous non-stimulated skin and of test histamine wheals in previous large histamine wheals of six healthy subjects. The wheals were measured with calliper on photographs of casts magnified on the screen of a computer. The test wheals of the 2 h experiment and of the 6 h experiment had no borders and therefore, heights of the test wheals were measured from diameters with the lengths of the diameters of the corresponding initial histamine wheals.

**FIGURE 3** Height of histamine test wheals in per cent of height of corresponding initial wheals (baseline) at different h after histamine skin prick tests had evoked large initial wheals in six healthy subjects. Results are given as mean values ± SD. Values below 100 indicate reduced sensitivity of histamine H1 receptors

all the initial wheals and of the 24 hours test wheals and 72 hours ones. The heights of the 18 hours test wheals were 3%-5% of their corresponding diameters, the heights of the 6 hours test wheals 2%-5%, and the heights of the 2 hours test wheals even lower. The diameters of the 18 hours test wheals were shorter than those of the 18 hours initial wheals ($P = .022$).

The results from arbitrary figures were found similar to those calculated in mm. Furthermore, areas of the 18 hours test wheals were smaller than areas of the 72 hours test wheals ($P = .046$). The measurements of the photographs, taken by the person with no knowledge of the aim of the study, showed almost the same results as those taken by the author. There was a difference between the heights of the test wheals of the 18 hours experiment and the 72 hours experiment both in mm ($P = .017$) and in arbitrary figures ($P = .036$).

TABLE 2 Heights of histamine wheals in previous allergen wheals

	Initial wheals	Test wheals	Difference	Change
2 h	.44 ± .16	.05 ± .04	$P = .001$	13 ± 11
6 h	.53 ± .08	.31 ± .08	$P = .003$	60 ± 15

Note: Mean heights ± SD of means in mm of initial histamine wheals in previous non-stimulated skin and of test histamine wheals in previous allergen wheals of six patients in each of the 2 h experiment and the 6 h experiment. The wheals were measured with calliper on photographs of casts magnified on the screen of a computer. The test wheals had no borders (except in one subject) and therefore, heights of the test wheals were measured from diameters with the length of the diameters of the corresponding initial histamine wheals. The column marked Change shows heights of test wheals in per cent of heights of initial wheals.

3.2 | The patients with allergen wheals

The allergen wheals were not circular. The mean diameter ± SD of the calculated diameters of the six patients of the 2 hours experiment was 10 ± 3 mm and of the 6 hours experiment 10 ± 2 mm.

All initial histamine wheals were circular and had distinct borders to the surrounding skin all around. None of the test wheals had borders except the wheal of one patient of the 6 hours experiment. Just before the 2 hours test histamine SPTs were set all the patients had some skin elevation there. The height of that elevation was subtracted from the height of the 2 hours test histamine wheal. No or very small skin elevations were observed just before the 6 hours test histamine SPTs were set. The heights and areas of the 2 hours test wheals or the 6 hours test wheals were lower than those of their corresponding initial histamine wheals. The results in mm from the calculations of the heights are seen in Table 2.

4 | DISCUSSION

The technique to take casts of human skin elevations is simple. The possibility to photograph and to measure the casts many times reduces the chance of subjective influences of the results. When a person with no knowledge of the aim of the present study took photographs of some of the casts, the results of the measurements differed very little from the results of measurements on photographs taken by the author.

When a histamine wheal with distinct borders is compared to a histamine wheal later evoked in the same, but in some way pre-treated, skin area, differences in magnitude and size must imply changes in histamine receptor sensitivity. Desensitized histamine receptors are described before in the literature. Smooth muscles made insensitive to histamine are well known since the method to estimate histamine amounts on pieces of guinea pig's jejunum. Sir John Henry Gaddum stated in a lecture in 1948 "If a high concentration is left in contact with the muscle the contraction is not maintained. The muscle relaxes, although still in contact with histamine; even after the solution has been changed the muscle remains for a

time insensitive to further applications of histamine...".² There are also other old publications describing reduced sensitivity of histamine receptors, mainly in animal experiments and inflammation. Human studies are very few. Greaves and Shuster described in 1967 tachyphylactic effect in the human skin to a second subcutaneous injection of histamine 35 minutes after the first one.³ The second injection in the same place as the first one did not evoke a wheal in six out of eight subjects.

H1 receptors are involved in the development of histamine wheals and allergen wheals. Stimulation of H1 receptors opens spaces between endothelial cells in small veins near the capillaries and causes dilatation of arterioli via release of neuropeptides. Contributing to the development of wheals are H1 receptor stimulated constriction of post-capillary resistance vessels. However, that is not mentioned in the literature as an important histamine effect on the development of histamine wheals. The present results indicate that H1 receptors with low sensitivity are found and that the time to restore the sensitivity of the receptors to normal is between 18 and 72 hours.

Total desensitization of H1 receptors was not demonstrated here. However, also low sensitivity may contribute to vasodilatation of post-capillary resistance vessels. Galajda et al have shown that histamine contributes via H1 receptors to the tone of venous vessels.⁴ Experiments were done on isolated veins from rabbits, but the authors suggested similar reactions in humans. There are some studies of isolated human veins indicating constriction when stimulated with histamine via H1 receptors.⁵ It is reasonable that human veins in vivo react in the same way. If that implies reduced tone and increased blood flow through post-capillary resistance vessels within histamine wheals, when H1 receptors are desensitized or have reduced sensitivity, is a reasonable speculation.

The allergic patients were histamine tested in the places where allergen SPTs were performed only 2 or 6 hours earlier. Similar results as with the large histamine wheals can be expected 18, 24 or 72 hours later. However, that can be discussed. Although no allergen wheals were seen by the naked eye 6 hours after the SPTs were set a late phase-reaction with influx of eosinophil leucocytes and certain lymphocytes exists.⁶ If that affects sensitivity of H1 receptors is not known.

There is a high prevalence of migraine in patients with allergic rhinitis compared with those without allergic rhinitis.⁷ Histamine as one cause to migraine is discussed since long and has recently been reviewed.⁸ The existence of clusters of meningeal mast cells adds the interest in histamine as an important substance in migraine. Then, can the findings in the present study be of help to understand how histaminergic migraine with aura starts and develops? A study of 952 migraine patients showed an average aura duration of 27 minutes.⁹ Histamine wheals increase in magnitude during around 18 minutes and are then slowly reduced.¹ The headache phase in migraine with aura lasts usually 1-2 days. The time for restoring the sensitivity of H1 receptors in the present study is between 18 and 72 hours. The Danish group around Olesen has demonstrated reduced blood flow during the aura phase and

increased flow during the headache phase of migraine.¹⁰ There is an old electron microscopic study speaking for reduced flow early in histamine wheals.¹¹ Subcutaneous histamine injections in rats evoked within five to ten minutes local constriction of veins, increased permeability, haemoconcentration and stasis in venules. Regarding vasodilatation in histamine wheals during the phase of re-sensitization there can only be speculation based on the studies of Galajda et al mentioned above.⁴ Other factors of uncertainty are the varying effects of histamine on blood vessels of different vascular regions both in vitro and in vivo. Furthermore, there are only few studies dealing with histamine receptors of cerebral or meningeal veins. However, it is tempting to suggest similar mechanisms behind histamine-evoked migraine with aura and mast cell-evoked or histamine-evoked wheal in the human skin.

To conclude, desensitization and re-sensitization of H1 receptors in the human skin are demonstrated which may explain some of the mechanisms in histaminergic migraine with aura.

ACKNOWLEDGEMENTS

The author is grateful to the nurses at the allergy reception of the Ear, Nose and Throat division of Skåne University Hospital, Sweden, for their help with histamine SPTs and allergen SPTs. This investigation was supported by a grant from the Royal Physiographic Society in Lund, Sweden.

CONFLICT OF INTEREST

The author declares no conflicts of interest.

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How to cite this article: Malm L. Re-sensitization of desensitized histamine H1 receptors in the human skin takes more than 18 hours. *Skin Res Technol*. 2019;00:1-6. <https://doi.org/10.1111/srt.12764>