

ORIGINAL PAPER

Influencing vascular reactivity *in vivo* by histaminergic agonists and antagonists

OANA ANDREIA COMAN¹⁾, C. ROTAR²⁾, SMARANDA STOLERU¹⁾,
ISABEL GHIȚĂ CRISTESCU¹⁾, H. PĂUNESCU¹⁾, I. FULGA¹⁾

¹⁾Department of Pharmacology

²⁾resident in Nuclear Medicine

"Carol Davila" University of Medicine and Pharmacy, Bucharest

Abstract

The eye is a target organ for the action of several topical or systemic drugs. The aim of the present study is to analyze the differences in reactivity between the iris and conjunctiva vessels after the topical administration of histamine and histamine receptor blockers respectively. Using a novel non-invasive technique for the quantification of the vascular diameters in the eye vessels, the response of these vessels to histamine, to H1 receptor blocker promethazine, and to H2 receptor blocker ranitidine versus vehicle (control) was analyzed. The results show differences in reactivity between iris and conjunctiva vascular territories. This data suggest that the population of histamine receptors differs between these two vascular areas.

Keywords: histamine, iris, conjunctiva, H1 receptor blockade, H2 receptor blockade, vascular diameter.

Introduction

Histamine is an endogenous amine secreted mainly by sensitized mast cells and present in almost all the tissues. Its role is due to the action on the four classes of histaminergic receptors [1, 2].

Stimulation of H1 receptors is responsible for bronchoconstriction and vasodilation [3, 4] and of H2 receptors for gastric chlorhydric acid hypersecretion and, to a lesser extent, for vasodilatation [5, 6].

The other two classes of receptors have been described recently. The H3 receptors function as presynaptic receptors with inhibitory effects on the histaminergic endings from the central nervous system (CNS) [7] and the H4 type is expressed in cells of hematopoietic lineage.

All types of receptors are coupled with G proteins (H1 is coupled with G_q, H2 with G_s, H3 and H4 with G_{i/o}) [1].

The vasodilatation induced by histamine is mediated by both H1 and H2 receptors, which have different localizations in the vascular bed [8].

The present study emerged from the consideration that if conjunctiva and iris vascular beds are of different embryological origins then it is possible that H1 and H2 receptors have different vascular localizations [8, 9].

The purpose of the study is to analyze the possible differences in reactivity between the iris and conjunctiva vessels after topical administration of histamine and histamine receptor blockers respectively. Literature data provide scarce information [10, 11] on this subject while new antihistaminic therapies are emerging for conjunctivitis treatment [12–14].

Material and methods

Male adult Wistar rats, weighting 250 g to 300 g (average 271 g), brought in the laboratory facilities a minimum of three days before the beginning of the experiment and kept on a standard diet were used. All the experiments were performed during daytime (12:00 to 16:00 hrs).

All rats were anaesthetized with chloralhydrate (produced by Redox) 20% 0.1 mL/100 g body weight) injected intraperitoneally and after five minutes pancuronium bromidum (Pavulon – Organon Holland) 0.02%, 0.1 mL/100 g body weight injected intraperitoneally was used to induce myorelaxation. Data recording was started after 10 minutes.

The image acquisition system was composed of a CCD camera (Toshiba IK–642E) and an AD converter interface (Pinnacle microVideo DC10+) connected to an IBM PC compatible system. The camera was fitted with a magnifying objective (Nikon) aided by an adapter (Navitar 1X Adapter 1–6015), allowing for resolutions within the optical microscopy range. Light was provided by a circular (ring-type fiber optics) source (Dolan–Jenner Industries Inc. model FiberLite series 180).

The camera was mounted on a holder (produced by IOR, Romania) allowing it to focus on the eye of the animal. The maximum optical resolution attained by the system was 12400 dpi (a pixel representing around 2×2 micrometers).

After immobilizing the animal, the optical system was adjusted manually until the image on the screen was adequately rich in blood vessels and its clarity was optimal.

For maximum accuracy, the lighting conditions and also the adjustment of the optical system were kept constant during the recording. In order not to induce experimental errors the image adjustment and acquisition was made for a single vascular area (iris or conjunctiva) for each animal.

A total of eight groups (six rats per group) were used as follows:

- Group 1 – distilled water (produced by Sicomed SA), 2 drops/eye, recording of the iris vessels for 300 seconds;

- Group 2 – histamine solution 1/1000 (w/v) (produced by Redox), 2 drops, recording of the iris vessels for 300 seconds;

- Group 3 – promethazine 25 mg/mL (Romergan, produced by Biofarm), 2 drops, recording of the iris vessels for 300 seconds; histamine (1/1000, 2 drops) was then administered starting from the second 300 and registration continued until 600 seconds;

- Group 4 – ranitidine 25 mg/mL (Zantac, produced by Glaxo Wellcome), 2 drops, recording of the iris vessels for 300 seconds; histamine (1/1000, 2 drops) was then administered starting from the second 300 and registration continued until 600 seconds;

- Group 5 – distilled water, 2 drops/eye, recording of the conjunctival vessels for 300 seconds;

- Group 6 – histamine solution 1/1000 (w/v), 2 drops, recording of the conjunctival vessels for 300 seconds;

- Group 7 – promethazine 25 mg/mL, 2 drops, recording of the conjunctival vessels for 300 seconds; histamine (1/1000, 2 drops) was then administered starting from the second 300 and registration continued until 600 s;

- Group 8 – ranitidine 25 mg/mL, 2 drops, recording of the conjunctival vessels for 300 seconds, histamine (1/1000, 2 drops) was then administered starting from the second 300 and registration continued until 600 s.

All substances given were conditioned in vials.

Figure 1 is a digital video recording of the eye vessels performed for each animal using VirtualDub 1.5.1 and Adobe PhotoShop 6.0. No video compression was used during the recording. No color or size alterations were performed. Measurements of the vascular diameter were performed at 0, 60, 200, 300 seconds (and also at 400 and 600 seconds for promethazine and ranitidine groups).

The first value of the vessel diameter (V_i), at moment 0, was considered a control value for each eye registration. The following values, considered actual values (V_a), were analyzed in relation with this initial value using the formula:

$$\frac{(V_i - V_a)}{V_i} \times 100$$

Simple specialized software was developed to effectively quantify the vascular diameter. This software allows the loading of a single frame of the recording. Only one image plane (green) is used because there are absorption peaks for hemoglobin in the spectral region corresponding to the green color and contrast is better in this color plane.

Figure 2 is a screenshot from our software.

The means of each eye values were compared with the control value (before the solutions' instillations) using a statistical significance t Student test.

Results

In the iris (Figure 3), the administration of histamine caused at 60 s an approximately 10% not statistically significant increase in the vascular diameter, which slightly lowered at 200 s and 300 s.

Promethazine decreased the vascular diameter by approximately 6% at 60 s; this decrease was higher at 200 s and reverts to approximately 6% at 300 s; the administration of histamine after promethazine did not produce any effect at 400 s. At 600 s, an 8% increase in vascular diameter occurred.

Ranitidine caused progressive vasoconstriction (with a maximum of 27% decrease in vascular diameter at 300 s). The administration of histamine after ranitidine resulted in an increase of the vascular diameter, relative to the value at 300 s. However, at 600 s the vascular diameter is still under the initial value (at 0 s).

In the conjunctiva (Figure 4), histamine caused progressive, statistically significant vasodilation at 60 s (10%), 200 s and 300 s (about 15%).

Promethazine did not cause any statistically significant changes in the vascular diameter at any moment. The administration of histamine after promethazine did not alter the vascular diameter at 400 s and 600 s.

Ranitidine did not cause any statistically significant changes in the vascular diameter, but the administration of histamine after ranitidine caused a 6% increase in the vascular diameter at 400 s. At 600 s, there was a 10% increase in the vascular diameter.

The control groups showed no changes in the vascular diameter for the 300s of recording, proving that the experimental method used in this trial is reproducible and that the results obtained are not due to a natural vascular reactivity but to the direct effect of the instilled drugs.

Discussions

In the iris, promethazine decreased the vascular diameter; the administration of histamine after promethazine produced a delayed not significant increase of this diameter (at 600 s). This fact could be explained by histamine acting on H2 receptors, which are known to produce vasodilatation with a slower onset. Ranitidine caused progressive statistic significant vasoconstriction. The administration of histamine after ranitidine caused rapid and constant increase in the vascular diameter relatively to moment 300 s. At 600 s, this vasodilatation however attained a negative value, which was not statistically different from zero, so the stimulation of H1 receptors by histamine did not counteract the effects of the H2 receptor blockade. The data suggest that *there is probably a histaminergic tonus in the iris, exerted dominantly through the H2 receptors.*

Figure 1 – Typical example of a frame from the video recordings. Iris vessels may be seen in the upper left corner. The larger oblique vessel in the middle of the image belongs to the conjunctiva and part of an anastomotic circle

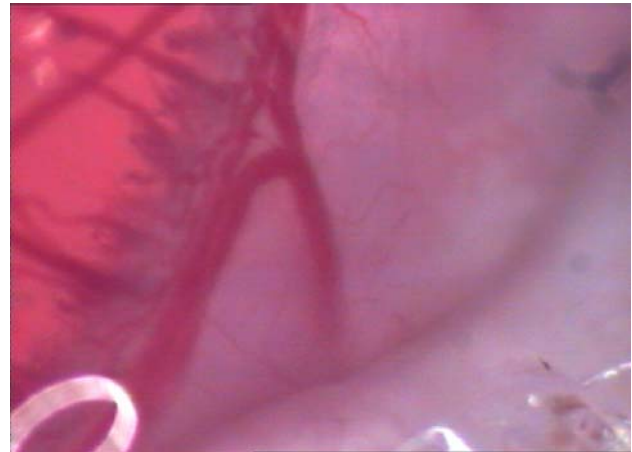


Figure 2 – Screenshot from the measurement software. In the middle of the image, the markers are placed so that the distance between them approximates the diameter of a larger conjunctival vessel. A histogram may be seen in the right corner

Figure 3 – Iris: variation of the relative vascular diameter (comparative with the initial state): “+” indicates $p < 0.05$ (single sample *t*-test) versus constant 0. Administration of histamine caused at 60 s an approximately 10%, not statistically significant increase in the vascular diameter, which slightly lowered at 200 s and 300 s. Promethazine decreased not significantly the vascular diameter (approx. 6%) between 60s and 300s; the administration of histamine after promethazine did not produce any effect at 400 s. At 600 s, a non-significant (8%) increase in vascular diameter occurred. Ranitidine caused progressive significant vasoconstriction (max. 27% decrease in vascular diameter at 300 s). The administration of histamine after ranitidine resulted in an increase of the vascular diameter, relative to the value at 300 s. However at 600 s the vascular diameter is still under the initial value (at 0 s)

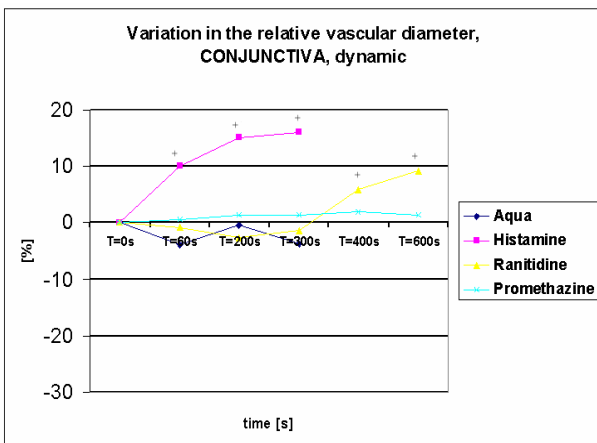
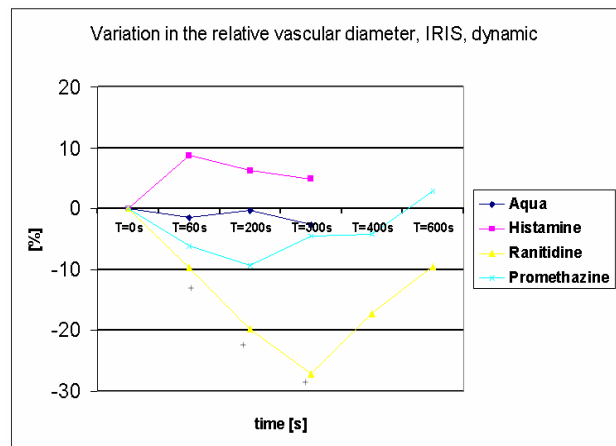


Figure 4 – Conjunctiva: variation of the relative vascular diameter (comparative with the initial state). “+” indicates $p < 0.05$ (single sample *t*-test) versus constant 0. Histamine caused progressive, statistically significant vasodilatation at 60 s (10%), 200 s and 300 s (about 15%). Promethazine did not cause any statistically significant changes in the vascular diameter at any moment. The administration of histamine after promethazine did not alter the vascular diameter at 400 s and 600 s. Ranitidine did not cause any statistically significant changes in the vascular diameter; the administration of histamine after ranitidine caused a 6% increase in the vascular diameter at 400 s. At 600 s there was a significant increase (10%) in the vascular diameter

In the conjunctiva, the administration of histamine caused progressive significant vasodilatation. Promethazine did not cause any changes in the vascular diameter at any moment.

The administration of histamine after promethazine did not alter the vascular diameter. This lack of effect may be due to the absence of H2 receptors in the conjunctiva. Ranitidine did not cause significant changes in the vascular caliber, but the administration of histamine after ranitidine caused an increase in the vascular diameter. This vasodilatation may be due to the action of histamine on the H1 receptors. Lack of effect of both histamine blockers suggest that *the histaminergic tonus is absent or diminished in the conjunctiva where the dominant receptor type may be H1*, according to the previous results.

In the present study, important differences were demonstrated between iris and conjunctiva vessels concerning two aspects: the histaminergic tonus is present in the iris vessels but seems to lack in the conjunctiva ones. On the other side, iris vessels seem to be rich in H2 receptors, while conjunctiva vessels seem to be rich in H1 receptors. Such differences were not signaled in the reviewed papers but could be important for future pharmacological approach of ophthalmologic pathology.

☐ Conclusions

In the present study a novel non-invasive technique for the quantification of vascular diameters has been developed. The data may suggest the existence of a histaminergic tonus in the iris, exerted primarily through the H2 receptors. The histaminergic tonus is absent or diminished in the conjunctiva, where the dominant receptor type is H1. Our findings clearly showed a difference in vascular reactivity between the iris and conjunctiva, manifested after administration of histaminergic agonists or antagonists.

✉ Corresponding author

Oana Andreia Coman, MD, PhD, Associate Professor at the "Carol Davila" University of Medicine and Pharmacy, Bucharest, Department of Pharmacology, Senior MD in Dermatology and Venereology, and in Clinical Pharmacology, 8 Eroilor Sanitari Avenue, 050 474 Bucharest, Romania; Phone +4021-318 07 62, E-mail: andreiacoman@yahoo.com

Received: September 11th, 2007

Accepted: November 10th, 2007

References

- [1] GOODMAN-GILLMAN A., HARDMAN J., LIMBIRD L., *Goodman & Gillman's, The Pharmacological Basis of Therapeutics*, 10th edition, McGraw-Hill Medical Publishing Division, New York, 2002, 581-600.
- [2] KATZUNG B. G., *Basic and clinical pharmacology*, Lange Medical Publication, Los Altos, California, 2002, 265-292.
- [3] BROGDEN R. N., MCTAVISH D., *Acrivastine. A review of its pharmacological properties and therapeutics efficacy in allergic rhinitis, urticaria and related disorders*, *Drugs*, 1991, 41(6):927-940.
- [4] DIACONU J. D., COMAN O. A., BENEVA V., *Tratat de terapeutică dermatovenerologică*, Ed. "Viața Medicală Românească", București, 2002, 128-139.
- [5] GANELLIN C. R., PARSONS M. E., *Pharmacology of histamine receptors*, Wright/PSG, Bristol, 1982, 298-322.
- [6] BURKHALTER A., JULIUS D., FRICK O., Histamine, serotonin and the ergot alkaloids. In: KATZUNG B. G. (ed), *Basic and clinical pharmacology*, 6th edition, Lange Prentice Hall Int., London, 1995, 251-275.
- [7] ISHIKAWA S., SPERELAKIS N., *A novel class (H3) of histamine receptors on perivascular nerve terminals*, *Nature*, 1987, 327(6118):158-160.
- [8] HILL S. J., *Distribution, properties and functional characteristics of three classes of histamine receptors*, *Pharmacol Rev*, 1990, 42(1):45-83.
- [9] ELLIS P., *Ocular therapeutics and pharmacology*, 6th edition, Mosby, St. Louis, 1981, 28.
- [10] MOLDERINGS G. J., WEISSENBOORN G., SCHLICKER E., LIKUNGU J., GÖTHERT M., *Inhibition of noradrenaline release from the sympathetic nerves of the human saphenous vein by presynaptic histamine H3 receptors*, *Naunyn Schmiedeberg's Arch Pharmacol*, 1992, 346(1):46-50.
- [11] SIMONS F. E., SIMONS K. J., *The pharmacology and use of H1-receptor-antagonist drugs*, *N Engl J Med*, 1994, 330(23):1663-1670.
- [12] FULGA I., COMAN O. A., CORCIOVEI CONSTANTINESCU I., CONSTANTINESCU C., ZUGRAVU A., GHIȚĂ CRISTESCU I., STOLERU S., COMAN L., ZUGRAVU G., *Farmacologie*, Ed. Medicală, București, 2004, 629.
- [13] STROESCU V., *Bazele farmacologice ale practicii medicale*, ediția a 5-a, Ed. Medicală, București, 1998, 650-651.
- [14] WATANABE T., YANAI K., *Studies on roles of the histaminergic neuron system by using pharmacological agents, knockout mice and positron emission tomography*, *Tohoku J Exp Med*, 2001, 195(4):197-217.