Studies of Spontaneous Fluctuations in Congestion and Nasal Mucosal Microcirculation and the Effects of Oxymetazoline Using Rhinostereometry and Micromanipulator Guided Laser Doppler Flowmetry

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ABSTRACT

The mucosa of the inferior turbinate was studied using rhinostereometry and micromanipulator-guided laser Doppler flowmetry in 10 healthy volunteers. First, spontaneous fluctuations were studied measuring congestion and multiple microcirculatory parameters simultaneously every 2 minutes. The subjects were then challenged with oxymetazoline using the same measuring technique studying the effects of the challenge during 12 minutes. There were spontaneous variations in congestion of up to 2.1 mm and variations in perfusion from 38% to 175% of average. There was no correlation between congestion in itself, or change in congestion, to perfusion or any other microcirculatory parameter. After challenge with oxymetazoline there was a rapid decrease in perfusion at 3 minutes after which there were no significant changes. The congestion decreased gradually throughout the procedure. Because congestion reflects the filling of the venous sinusoids and the flowmetry the state of the superficial vessels, we conclude that there are spontaneous short-term fluctuations in the sympathetic tone with independent actions on the different vessels. After challenge with a sympathomimetic drug, there was a decrease in both swelling and flow, but not synchronized. The combination of rhinostereometry and micromanipulator-guided laser Doppler flowmetry is a useful tool to study the dynamics of intranasal challenge reactions. (American Journal of Rhinology 13, 1–6, 1999)

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In most types of rhinitis, the major presenting symptom is difficulty in breathing through the nose due to nasal congestion. Congestion of the nasal mucosa is caused by changes of the mucosal microcirculation and is often treated with drugs acting on the vessels. To understand the physiology and pathophysiology of rhinitis as well as pharmacological effects on the mucosa, it is important to have a method of studying the relation between nasal mucosal congestion and the microcirculatory events in the mucosa underlying the changes in congestion.

Laser-Doppler flowmetry (LDF) is the only noninvasive method for studying microcirculation and has the advantage of giving continuous and instantaneous measurements of nasal mucosal blood flow.[1,2] Noninvasiveness is of great importance to avoid artifacts[3] It can also provide multiple microcirculatory parameters, i.e., the concentration of moving blood cells (CMBC), the velocity of the blood flow and the product of these two, perfusion or flow. The pulsatility of flow can also be evaluated. Some investigators have presented the laser Doppler registrations from the nasal mucosa in quantitative terms, i.e., as mL/100 g tissue/min[4] Because of the lack of calibrations against other methods for measuring blood flow in the specific tissue, we prefer to express the data in arbitrary units, perfusion units (PU), concentration units (CU), and velocity units (VU).

An optical high precision method of measuring changes in mucosal congestion, rhinostereometry (RSM) has been devised by Juto.[5]

The combination of LDF and RSM has the advantage of using two noninvasive methods for direct and simultaneous measurements of congestion and microcirculation because rhinostereometry allows the laser probe to be in place in the nose during the measuring of congestion. The same spot on the mucosa is measured. It also allows for a high degree of control of the position of the probe.

In a study using RSM and LDF on intranasal challenge with histamine in increasing concentrations, it was possible to monitor simultaneously the changes in congestion and microcirculation.[6] The reactions seemed roughly to correspond to the different vascular layers in the mucosa, reflecting the action of histamine as it penetrated into the mucosa from the surface on down.

In another study, we evaluated the importance of the measuring distance from the laser Doppler probe to the surface of the mucosa.[7] In this study, we used a micromanipulator to position the probe tip under microscopic visual control at a well defined distance from the mucosa. We found that the LDF readings were sensitive to changes in distance down to 0.1 mm. The parameters of perfusion and velocity dropped significantly when the probe was moved away from the surface. We proposed that a suitable measuring distance should be 0.3 mm, because the readings at this distance did not significantly differ from the values obtained at contact with the mucosa. At this distance it is also easier to avoid touching the mucosa with the probe.

It is well known that the nasal mucosa undergoes spontaneous variations in congestion. The nasal blood vessels receive a dense sympathetic innervation, and stimulation of the sympathetic nerves to the nose cause a reduction of nasal blood flow[8] and a decongestion of nasal venous erectile tissue.[9–11] Interruption of the resting sympathetic tone by nerve section or local anesthesia of the stellate ganglion causes congestion due to swelling of the venous tissue.[12,13] An important manifestation of the sympathetic tone to the nasal venous tissue is the nasal cycle as described by Eccles.[14] Änggård, among others, has shown that different frequencies in sympathetic nerve stimulation caused different reductions in tracer disappearance rates and in local blood content.[9]

When studying variations in nasal mucosa congestion during rest using RSM, Juto and Lundberg found variations in congestion between the volunteers ranging within 2–3 mm during 4 hours.[15]

In another study with RSM on the effects of sympathetic stimulation induced by physical exercise, there was a profound decongestion followed by a rapid recongestion.[16] Ohki et al. studied the effects of exercise on nasal airway resistance measured by rhinomanometry and nasal blood flow by LDF.[17] There were decreases of both immediately after exercise, but the rates of changes were not correlated to each other and there was a discrepancy between resistance and flow during the latter period after exercise. They concluded that since LDF measures blood flow in the superficial parts of the nasal mucosa, the vessels in the various parts do not always respond in the same way.

However, during RSM measurements it is obvious that in most subjects there are pronounced short term variations in congestion, especially during the acclimatization period that is normally allowed to precede the actual RSM procedure. The object of this study was to use the combination of RSM and LDF to determine whether these fluctuations in congestion were correlated to fluctuations in the LDF parameters. We also wanted to compare these spontaneous fluctuations in congestion and microcirculation to changes elicited by intranasal challenge with a well known drug, oxymetazoline, mimicking an increase in the sympathetic tone on the nasal vessels by acting on the α 2-adrenergic receptors.

SUBJECTS AND METHODS

RSM is a direct optical method where the subject is secured to a frame by means of an individually adapted dental splint. The frame holds a micrometer table carrying a surgical microscope with a small depth of focus. The micrometer table is equipped with four microgauges, three of which define the position of the microscope along the perpendicular dimensions up-down, anterioposterior and right to left and the fourth the rotation around a vertical axis. The eye-piece is equipped with a horizontal millimeter scale. The position of the surface of the mucosa is determined by defining the point at which it intersects a horizontal line in the frontal plane defined as the intersection of the plane of focus of the microscope and the horizontal plane defined by the millimeter scale. Changes of the mucosal surface position greater than 0.18 mm can be determined between measurements on different occasions in the same subject according to Juto and Lundberg.[18]

LDF was performed using a Periflux 4001 (Perimed, Sweden). The wave-length of the laser beam was 780 nm. A specially designed probe was used with an outer diameter of 1.6 mm and a fiber separation of 0.5 mm. The surface of the probe end was angled 15 degrees from the line of sight in order to keep this surface parallel to the mucosa.

An RSM apparatus equipped with a micromanipulator (Rhinomed, Sweden) was used to combine the two methods. Thus it was possible to position, and if the mucosal swelling altered, continuously adjust the laser probe end to the desired distance to the mucosal surface to be studied. The probe is placed in the plane of focus and can then be adjusted in the dimensions up-down and side to side with an accuracy of 0.1 mm. Should the surface diverge from the vertical plane, the probe can be turned around its axis in order to obtain parallelity. In this way it was possible to keep the measuring distance stable during the registration of flowmetry values. The signal was fed into a IBM compatible computer using the PERISOFT software program.

In the study of spontaneous fluctuation nine healthy volunteers participated. They were not allowed to acclimatize and were immediately placed in the RSM apparatus. Thereafter the degree of congestion according to RSM and the LDF parameters perfusion, CMBC and the velocity of flow were measured simultaneously every 2 minutes, in each subject a total number of 8 times, i.e., during 14 minutes. Each measurement was performed within 30 seconds. The subjects were allowed to leave the apparatus between measurements. The site of measuring was the anteromedial surface of the inferior turbinate. The probe was placed every time at a distance of 0.3 mm from the surface of the mucosa.

In the study of oxymetazoline 10 healthy volunteers participated. These were the same subjects as in the previous study, with one additional volunteer. They were allowed to acclimatize over 15 minutes. Thereafter the degree of congestion according to RSM and the LDF parameters perfusion, concentration and velocity were measured. Immediately thereafter they were challenged with one spray-dose of oxymetazoline 0.5 mg/mL equal to 0.025 mg into the right nostril. The congestion and the LDF parameters were measured simultaneously after 3, 6, 9, and 12 minutes. As before each measurement was performed within 30 seconds and the subjects were allowed to leave the apparatus between measurements. The coordinates of the stereometer were the same as in the fluctuation study. Therefore the same spot on the mucosa was measured in the nine subjects who took part in both studies. The probe was placed every time at a distance of 0.3 mm from the surface of the mucosa.

In order to study correlation between spontaneous fluctuation in congestion and microcirculation, we first calculated the change in congestion in each individual from each occasion to 2 and 4 minutes later. In order to compare the subjects as a group, the LDF parameters perfusion, CMBC, and velocity for each occasion were then divided with the mean value for each subject and expressed as a percentage of the average of all readings of the respective parameter in that subject. Each percentual value of the LDF parameters was then analyzed for correlation to change in congestion during the 2 and 4 minutes after the LDF reading, and for 2 minutes before to 2 minutes after the LDF reading. The analysis of correlations was made using the Spearman rank correlation test.

In the study of oxymetazoline, the mean and SEM for the group was calculated for congestion, perfusion, CMBC, and velocity before and 3, 6, 9, and 12 minutes after challenge. Significance in changes were calculated using the Wilcoxon signed rank test.

RESULTS

All individuals completed the study. There were no adverse events and all measurements could be performed as planned.

Fluctuation

During the measuring process of 14 minutes there were spontaneous variations in swelling in the group in the range of up to 2.1 mm. In the LDF readings there were spontaneous variations in perfusion from 38% to 175%, in CMBC from 81% to 123%, and in velocity from 45% to 170% of average in each individual. According to the Spearman rank correlation test there was, however, no correlation between any of the LDF parameters on one hand, and the degree of swelling in itself or change in swelling on the other hand. This was true for all of the time intervals described above.

Oxymetazoline Challenge

In the challenge test with oxymetazoline there was a continuous decongestion throughout the measuring period. The changes were significant from 0 to 3 minutes ($p < 0.008$) (Fig. 1)

There was a significant decrease in perfusion from 0 to 3 minutes ($p < 0.02$). After that, there was no further decrease in perfusion (Fig. 2).

The CMBC was almost unchanged throughout the challenge. The only significant change was a decrease from 9 to 12 minutes ($p < 0.04$).

Since the CMBC was almost unchanged, and the velocity is the perfusion divided by the CMBC, the velocity changed in a pattern similar to that of perfusion. From 0 to 3 minutes there was a significant decrease ($p < 0.012$). After that, there was no further decrease in velocity (Fig. 3).

DISCUSSION

When studying changes in nasal congestion and microcirculation especially during intranasal challenges with drugs having rapid effects on both, it is an advantage to use a combination of methods that allows for real-time simultaneous measuring of congestion and microcirculation. Changing equipment between different types of measuring should be avoided, since it causes delays in time and subjects the patients to manipulation that can cause reactions in the nasal mucosa. Both methods should be noninvasive. It is also an advantage to perform both measuring techniques on the same portion of the nasal mucosa, in order to more closely relate changes in congestion and microcir-

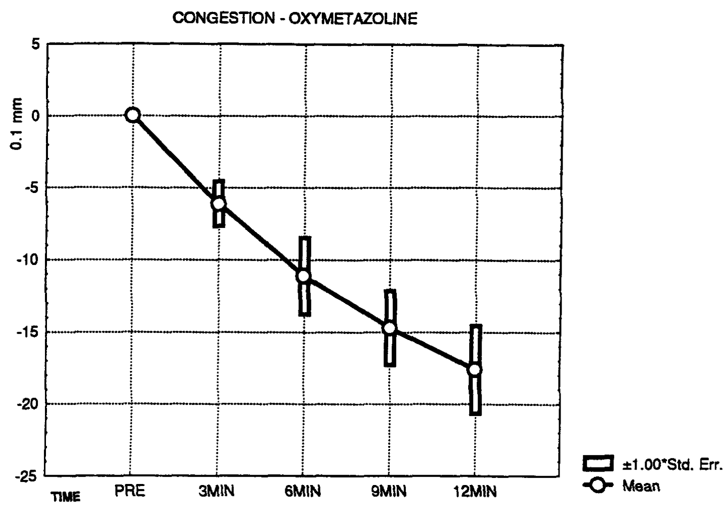

Figure 1. Changes in congestion after challenge with oxymetazoline. The changes are significant from 0 to 3 minutes ($p < 0.08$), from 3 to 6 minutes ($p < 0.01$), from 6 to 9 minutes there is a borderline significance ($p < 0.09$) and from 9 to 12 minutes the change was significant ($p < 0.018$).

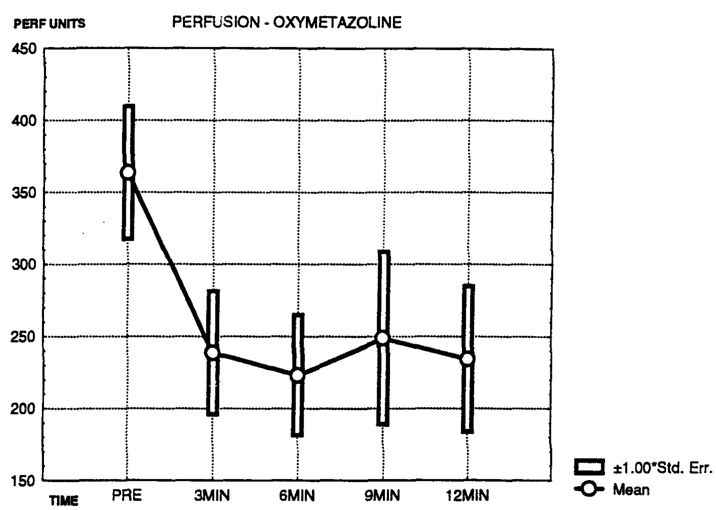

Figure 2. Changes in perfusion after challenge with oxymetazoline. The decrease from 0 to 3 minutes is significant ($p < 0.02$). The other changes are not significant, showing no further decrease.

culation in that specific part of the mucosa. RSM is a direct optical method for assessing nasal mucosal congestion, having the advantage of visual observation of the measured portion of the mucosa throughout the procedure. One disadvantage is that the subject has to bite on an individually fitted tooth splint. Prolonged biting can cause stiffness and tension in the jaws and neck and therefore result in stress reactions affecting the nasal mucosa. This can be avoided by letting the subject out of the apparatus between the measurements. Because the positions of both the subject and the microscope are precisely defined by the splint and the microgauges, the same position can always be reached. With the subject in place in the rhinostereometer, it is possible to use a micromanipulator to place the LDF probe in an exact position at a desired distance from the surface of the mucosa at the same spot being used for measuring

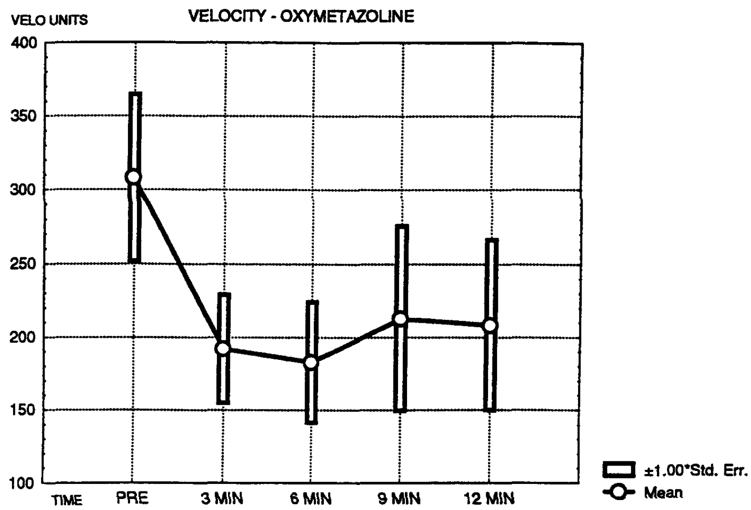

Figure 3. *Changes in velocity after challenge with oxymetazoline. The decrease from 0 to 3 minutes is significant ($p < 0.012$). The other changes are not significant, showing no further decrease.*

congestion. Both the mucosal surface and the probe are then viewed with a linear magnification of 18 times.[7] Because the process of repositioning the subject in the splint and placing the probe does not exceed 30 seconds, it is possible to allow the subject adequate rest between measurements performed down to every minute if desired.

The advantage of studying multiple parameters has been pointed out by Druce et al.[19]

Unlike some investigators who have chosen the septum for recording LDF, we have chosen the inferior turbinate because we believe that this mucosa is more sensitive to variations in sympathetic tone. The anteromedial aspect of the inferior turbinate is located in the narrowest part of the nasal airway passage.[20]

In the fluctuation study the measurements were repeated every 2 minutes. The setup conditions were as stable as possible, the same spot on the mucosa being measured and the LDF probe placed in position without touching the mucosa at the same distance of 0.3 mm each time. Despite the fact that the subjects were healthy, without symptoms of nasal hyperreactivity, and not subjected to any drugs, there were quite pronounced variations in congestion during the 14 minutes of registration in each subject. This is plausible due to the absence of an acclimatization period. There were also large variations in perfusion and velocity. The concentration readings were much more stable. There was no statistical correlation between either congestion in itself or change in congestion to the LDF parameters. RSM measures the congestive status of the mucosa, probably mainly reflecting the degree of filling of the venous sinusoids[21] and the LDF parameters reflect mainly the flow in the more superficial layers of the mucosa, containing arterioles and a dense subepithelial capillary network.[22] The results show that there are spontaneous short-term fluctuations in both types of vessels but that they do not correlate with each other. This could therefore suggest that there is a dissociation in the variation of sympathetic tone acting on the different types of vessels. This could relate to the findings of Ännggård concerning the effects of different frequencies in sympathetic nerve stimulation.[9]

Cauna has suggested that the control of nasal mucosal congestion may depend on the balance between the filling of the tissue via arteriovenous anastomoses and the drainage of the tissue depending on the activity of specialized cushion or throttle veins. It is proposed that LDF measures the superficial layers of the nasal mucosa and does not reflect the status of the deeper situated venous sinusoids.[24] However, using RSM to evaluate the congestive status of the same portion of mucosa being measured with LDF, it is possible to relate changes in the vascular beds to each other. The activity of the deep situated throttle veins can not be seen with LDF but can be indirectly assessed by using RSM.

We also wished to relate the spontaneous changes in congestion and microcirculation to the immediate changes caused by a well known drug mimicking an increase in the sympathetic tone by acting on the α 2-adrenergic receptors. Wight and Cochrane studied the effects of a similar substance, xylometazoline on LDF and nasal airway resistance measured by rhinomanometry.[25] They found significant decreases in both nasal resistance and blood flow. They found, however, no correlation between the percentage changes in airflow and blood flow and concluded, like Ohki et al,[17] that the two types of measurements reflect different parts of the nasal vascular bed. In the study of oxymetazoline there was a rapid effect on the arterial circulation. The perfusion dropped significantly after 3 minutes. After that no significant further decreases were observed. The congestion, on the other hand, showed a moderate decrease after 3 minutes, the decrease continuously progressing during the measuring period of 12 minutes. These differences in reaction over time can probably be explained by the diffusion or transportation of the drug down from the surface, acting on different layers of the mucosa in sequence. A similar pattern of increases in congestion and microcirculation was seen in our previous study on histamine.[6]

CONCLUSION

Using the combination of RSM and LDF it was possible to determine spontaneous fluctuations in both congestion and microcirculation, which show no correlation with each other. When challenging the subjects with oxymetazoline, there was a uniform reaction within the group. The perfusion showed a rapid decrease after 3 minutes and did not change after that. The congestion, on the other hand, showed a gradual decrease throughout the study. When trying to relate effects of intranasal challenges on congestion and microcirculation to each other, it therefore seems necessary to use a combination of methods that allow for quick and repeated simultaneous measurements of both variables in order to detect the dynamics of the effects on different vascular components.

REFERENCES

1. Tenland T. On laser Doppler flowmetry. Methods and microvascular applications. Thesis. University of Linköping, Sweden 1982.
2. Olsson P, Bende M, Ohlin P. The laser Doppler flowmeter for measuring microcirculation in human nasal mucosa. Acta Otolaryngologica 99:133–139, 1985.
3. Druce HM. Nasal blood flow. Ann Allergy 71:288–291, 1993.
4. Druce HM, Bonner RF, Patow C, et al. Response of nasal blood flow to neurohormones as measured by laser Doppler velocimetry. J Appl Physiol 57:1276–1283, 1984.
5. Juto JE. Rhinostereometry. Thesis, Karolinska Institute, Stockholm, 1985.
6. Grudemo H, Juto JE. Rhinostereometry and laser-Doppler flowmetry in human nasal mucosa. Changes in congestion and microcirculation during intranasal histamine challenge. ORL 59:50–56, 1997.
7. Grudemo H, Juto JE. The impact of the measuring distance on the laser-Doppler measurements of the microcirculation in human nasal mucosa. A study of rhinostereometry and micromanipulator-guided laser Doppler flowmetry. ORL 59:280–285, 1997.
8. Änggåard A, Densert O. Adrenergic innervation of the nasal mucosa in the cat. Acta Otolaryngol 78:232–241, 1974.
9. Änggåard A, Edwall L. The effect of sympathetic nerve stimulation on tracer disappearance rate and local blood content in the nasal mucosa of the cat. Acta Otolaryngol 77:131–139, 1974.
10. Malm L. Stimulation of sympathetic nerve fibres to the nose in cats. Acta Otolaryngol 75:519–526, 1973.
11. Eccles R, Wilson H. The autonomic innervation of the blood vessels of the cat. J Physiol 238:549–560, 1974.
12. Stoksted P, Thomsen KA. Changes in the nasal cycle under stellate ganglion blockade. Acta Otolaryngol 109:176–181, 1953.
13. Rooker DW, Jackson RT. The effects of certain drugs, cervical sympathetic stimulation and section on nasal patency. Ann Otol Rhinol Laryngol 78:403–414, 1969.
14. Eccles R. The central rhythm of the nasal cycle. Acta Otolaryngol 86:464–468, 1978.
15. Juto JE, Lundberg C. Variation in nasal mucosa congestion during rest. Acta Otolaryngol 98:136–139, 1984.
16. Juto JE, Lundberg C. Nasal mucosa reaction, catecholamines and lactate during physical exercise. Acta Otolaryngol 98:533–542. 1984.
17. Ohki M, Hasegawa M, Kurita N, Watanabe I. Effects of exercise on nasal resistance and nasal blood flow. Acta Otolaryngol 104: 328–333, 1987.
18. Juto JE, Lundberg C. An Optical method for determining changes in mucosal congestion in the nose in man. Acta Otolaryngol 94:149–156, 1982.
19. Druce HM, Kaliner MA, Ramos D, Bonner RF. Measurement of multiple microcirculatory parameters in human nasal mucosa using laser Doppler velocimetry. Microvasc Res 38:175–185, 1989.
20. Hilberg O, Grymer LF, Pedersen OF, Elbrond O. Turbinate hypertrophy, evaluation of the nasal cavity by acoustic rhinometry. Arch Otolaryngol Head Neck Surg 116:283–289, 1990.
21. Malm L, Druce HM, Holgate ST. Vasoconstrictors and antihistamines. In Allergic and Vasomotor Rhinitis: Clinical Aspects. Mygind N, Weeks B, Eds. Copenhagen: Munksgaard, 140–150. 1985.
22. Cauna N. Blood and nerve supply of the nasal lining. In The nose: Upper airway physiology and the atmospheric environment. Proctor DF, Andersen JB, Eds. Elsevier Biomed Press, 45–69, 1982.
23. Cauna N, Cauna D. The fine structure and innervation of the cushion veins of the human nasal respiratory mucosa. Anat Rec 181:1–16, 1975.
24. Olsson P. A comparison between the 133Xe washout and laser Doppler techniques for estimation of nasal mucosal blood flow in humans. Acta Otolaryngol 102:106–112, 1986.
25. Wight RG, Cochrane T.A comparison of the effects of xylometazoline on nasal airflow, and on blood flux as measured by laser Doppler flowmetry. Acta Otolaryngol 108:284–289, 1989. □