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Enhanced presence of serotonin in nasal cavity after autologous stimulation

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Abstract. – OBJECTIVE: Serotonin, which is a vasoactive amine, is an important neurotransmitter and is involved in many behavioral and psychological phenomena, such as pain, appetite, mood, and sleep. The primary purpose of our study was to investigate the effect of high-pressure administration of sterile physiological saline isotonic solution (HpPSIS) into nasal cavity and to determine the expression of the serotonin.

PATIENTS AND METHODS: The study was made in two branches, the previous with 14 volunteers, the subsequent study with 40 patients with mild anxiety disorder. The middle third of the inferior turbinate epithelial cells on the right nostril was scraped using a sterile curette and indicated as (pre), then, a spray of sterilized isotonic solution at high pressure on the left nostril was delivered, and 5 minutes later a similar stimulation was delivered on the same nostril. The stimulation was made with a specific spray dispenser. The middle third of the inferior turbinate epithelial cells on the left nostril was scraped using a sterile curette and indicated as (post). Then, based on the first part of our study, we started the second part and gave a treatment on forty new patients with anxiety disorder.

RESULTS: The results of these studies highlight the possibility of endogenous enhancement of serotonin by stimulation of mast cells. In the first part of the study, Serotonin significantly increased in protein extracts after treatment (64.35±5.33 *vs.* 10.97±2.17; unpaired two tailed *t*-test, t=9.8, df=24, $p \leq 0.0001$; F=6.035; DFn=12; DFd=12). In the second part of the study, in patients treated with HpPSIS, we observed improvement of mood, after one, two and three months, with a statistically significant reduction of DASS-21, while no reduction was observed in control patients, treated with normal pressure commercial spray.

CONCLUSIONS: This pilot study showed that the topical treatment of HpPHIS increases serotonin levels in nasal cavity. The observation reported in this study opens the way to a new valid strategy to enhance the level of endogenous serotonin. We observed a significant improvement of ASI on patients during HpPHIS therapy.

Key Words:
NGF, Seronotine, High-pressure physiological hypotonic saline solution (HpPHIS).

Introduction

In a previous study, we observed that autologous stimulation, induced by nasal forced stress with an isotonic solution, causes an increase in mast cells with a release of NGF in the nasal cavity[1]. The presence of mast cells and their degranulation has a broad implication for understanding the role in wound healing and peripheral nerve repair in the nasal mucosa and most probably in clinical prospective. We, then, demonstrated that nasal administration of high-pressure physiological hypotonic saline solution (HpPHIS) stimulates the production and release of NGF from cells in the nasal cavity of laboratory mice and it reaches the forebrain[2]. We have also shown that HpPHIS administration, through olfactory pathways, also induces the release of NGF and upregulation of NGF receptors in patients affected by brain tumors, while reducing the number of brain tumor cells by stimulating the release of the anti-tumor protein p73[3]. The primary purpose of our study is to determine the expression of the serotonin nasal presence. A large number of studies have focused on the role of serotonin as a neurotransmitter in the central nervous system, although only a small percentage of the body's serotonin (~ 5%) can be found in the mature brain of mammals[4,5].

Serotonin, which is a vasoactive amine, is an important neurotransmitter and is involved in many behavioral and psychological phenomena, such as pain, appetite, mood, and sleep[6]. Addition-

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ally, serotonin has immunomodulatory activities that occur *via* serotonergic receptors activation[7]. Serotonergic receptors have been characterized in lymphocytes, monocytes, macrophages, and dendritic cells. These receptors are divided into seven classes based on their structures and have been found to cause biologic effects *via* different signal-transduction pathways. Recent studies[8] have shown the expression of 5-HT7 receptors in the nasal cavity on inferior turbinate.

Patients and Methods

First Part

The study was made in two branches, the previous with 14 volunteers, the subsequent study with 40 patients with mild anxiety disorder. The Ethical Committee of the hospital (Campus Bio-Medico University of Rome) approved the study and informed consent for tissue analysis was obtained from all the patients. Patients with nasal polyposis, chronic rhinosinusitis, ongoing pregnancy, smokers, and nosebleeds, patients who already underwent nasal surgery, patients with marked septal deviation and or turbinate hypertrophy, patients immunocompromised, and patients with bronchial asthma or chronic obstructive pulmonary disease (COPD), patients who had used antibiotics in the previous 30 days, patients that chronically use immunosuppressive corticosteroid, were excluded.

The middle third of the inferior turbinate epithelial cells on the right nostril was scraped using a sterile curette and indicated as (pre), then, a spray of sterilized isotonic solution at high pressure on the left nostril was delivered, and 5 minutes later a similar stimulation was delivered on the same nostril. The stimulation was made with a specific spray dispenser. The middle third of the inferior turbinate epithelial cells on the left nostril was scraped using a sterile curette and indicated as (post).

Serotonin quantification (ELISA)

Serotonin protein was measured in nasal scraping by using a commercially available ELISA kit (Serotonin ELISA kit; ADI-900-175; Enzo Life Sciences; Ann Arbor, MI, USA and ab133053, Abcam, Waltham, MA, USA), according to the manufacturer's instructions with minor modifications. Briefly, nasal scraping swab were immersed in 100 µl modified RIPA buffer (50 mM tris-HCl, pH 7.5; 150 mM NaCl; 5 mM EDTA; 1% Triton X-100; 0.1% SDS; 1 mM PMSF and 1x protease inhibitors Pierce) and crude extracts were centrifuged at 13000 rpm for 15 min to remove debris. The supernatant was collected for Serotonin detection. Appropriate sample extraction and dilutions were carried out in lysis buffer and sample diluent (1:2 diluted). Diluted samples were loaded on 96-well pre-coated plates in parallel with standard curves (range 0.49-500 ng/ml). Absorbance (OD) values were recorded after reading the plates at λ450 nm (corrected to λ570 nm) in a 96-well plate reader platform (Sunrise; Tecan Group Ltd., Männedorf, Switzerland). Normalization was carried out before assay. The sensitivity of assay was estimated as 0.293 ng/ml and the average intra/inter-assay coefficient of variations (CV) were r espectively <10% and <12%.

Second Part

In the first part of the study, we observed an increase of the serotonin levels. So based on the first part of our study, we started the second part and gave a treatment on forty new patients with anxiety disorder. The patients were divided in two groups of 20 patients; the first one was treated for three months with HpPSHS 7 gr/sec for 0.2 sec ET (emission time) on both nostrils, 5 minutes later a similar stimulation was delivered twice a day; and the control group received second one with low pressure nasal spray, of common use in the pharmaceutical market, 1 gr/sec for 0.2 sec.

Depression, Anxiety and Stress Scale-21 (DASS-21) (Figure 1) was filled out before treatment and after one, two and three months[9,10]. The instructions ask the participant to rate on a 4-point scale how often the described experience applies to him/her in general (e.g., "How often do……", "you feel rested", "you have many things to do", "you feel frustrated"). Response options are on a 4-point scale (0 = did not apply to me at all and 3 = applied to me most of the time). Higher scores indicate more psychological distress[11].

After three months of therapy, the first group was divided in two groups of ten patients, the first one continued the HpPSIS, while the other shifted to treatment with low pressure nasal spray. After one month, DASS-21was filled out by patients of both groups.

Statistical Analysis

All values were expressed as mean±SEM in the graphics. Unpaired Student t test analysis was performed using the StatView software (Abacus Concepts Inc., Barkley, CA, USA). A p-value ≤ 0.05 was considered significant. All graphs were performed using GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA, USA).

DASS21 *Name:* *Date:*

Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you *over the past week*. There are no right or wrong answers. Do not spend too much time on any statement.

The rating scale is as follows:

0 Did not apply to me at all
1 Applied to me to some degree, or some of the time
2 Applied to me to a considerable degree, or a good part of time
3 Applied to me very much, or most of the time

1	I found it hard to wind down	0	1	2	3
2	I was aware of dryness of my mouth	0	1	2	3
3	I couldn't seem to experience any positive feeling at all	0	1	2	3
4	I experienced breathing difficulty (eg, excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3
5	I found it difficult to work up the initiative to do things	0	1	2	3
6	I tended to over-react to situations	0	1	2	3
7	I experienced trembling (eg, in the hands)	0	1	2	3
8	I felt that I was using a lot of nervous energy	0	1	2	3
9	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3
10	I felt that I had nothing to look forward to	0	1	2	3
11	I found myself getting agitated	0	1	2	3
12	I found it difficult to relax	0	1	2	3
13	I felt down-hearted and blue	0	1	2	3
14	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3
15	I felt I was close to panic	0	1	2	3
16	I was unable to become enthusiastic about anything	0	1	2	3
17	I felt I wasn't worth much as a person	0	1	2	3
18	I felt that I was rather touchy	0	1	2	3
19	I was aware of the action of my heart in the absence of physical exertion (eg, sense of heart rate increase, heart missing a beat)	0	1	2	3
20	I felt scared without any good reason	0	1	2	3
21	I felt that life was meaningless	0	1	2	3

	Depression	Anxiety	Stress
Normal	0-9	0-7	0-14
Mild	10-13	8-9	15-18
Moderate	14-20	10-14	19-25
Severe	21-27	15-19	26-33
Extremely Severe	28+	20+	34+

Figure 1. Depression, Anxiety and Stress Scale-21 (DASS-21). Test and reference values.

Results

First Part

Serotonin was quantified in nasal scraping collected before and after treatment. As shown in Figure 2A, Serotonin significantly increased in protein extracts after treatment (64.35±5.33 vs. 10.97±2.17; unpaired two tailed *t*-test, t=9.8, df=24, $p \leq 0.0001$; F=6.035; DFn=12; DFd=12). Scatter plot in Figure 2B indicate the expression from each individual subjects.

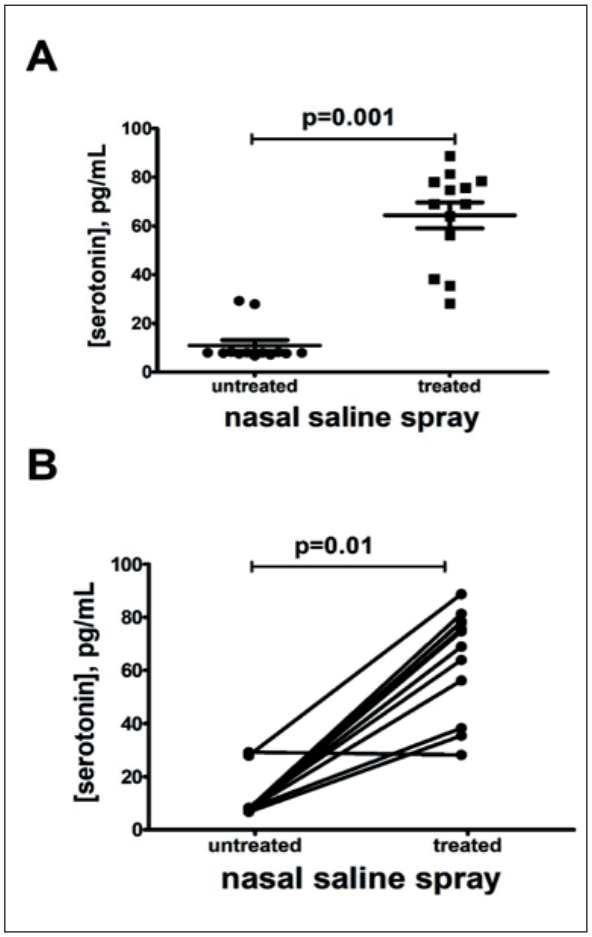

Figure 2. A-B, Serotonin expression in nasal scraping before and after treatment with high-pressure hypotonic saline solution. **A,** Comparison of serotonin protein expression in nasal scraping from treated nostril *vs.* untreated nostril. Statistical significance between subgroups is shown in the graph. **B,** Scatter plots comparing the serotonin expression in nasal scraping from each nostril.

Second Part

In patients treated with HpPSIS we observed improvement of mood, after one, two and three months, with a statistically significant reduction of DASS-21, while no reduction was observed in control patients, treated with normal pressure commercial spray (Figure 3A). After three months we divided the patients treated with HPNS in two groups of ten patients: the first one continued the HpPSIS therapy for one month and the second one continued with a normal spray with low pressure output. At the end of treatment, the first group showed an ASI score similar to those during treatment, with not significant variations, while the second group had a significant worsening of the mood, with a statistically significant increase of the ASI score. The single and overall score of the second group returned to the score registered at the pretreatment enrollment, with not significant variations (Figure 3B).

Discussion

Mast cells (MCs) are derived from hemopoietic precursor cells, undergo their maturation in peripheral tissues, and play a significant role in both the innate and adaptive immune response. Serotonin is not stored in all MC types but is implicated in MC adhesion, chemotaxis, and tissue regeneration through smooth muscle differentiation of stromal cells. With this study we have shown that nasal MCs release serotonin. A recent study detailed 5-HT expression in inferior turbinate in patients with nasal polyps resulting in increased serotonin levels. In previous works we demonstrated that an inflammatory reaction due to HpPHIS can lead to recruit Mast cells, and subsequently trigger the release of granules and their contents, i.e., NGF. It was feasible that the same process happened with serotonin, which is also present in MC granules, and in this work, we confirmed the hypothesis, observing increase of serotonin nasal levels after MC induced degranulation.

This pilot study showed that the topical treatment of HpPHIS increases serotonin levels in nasal cavity. The observation reported in this

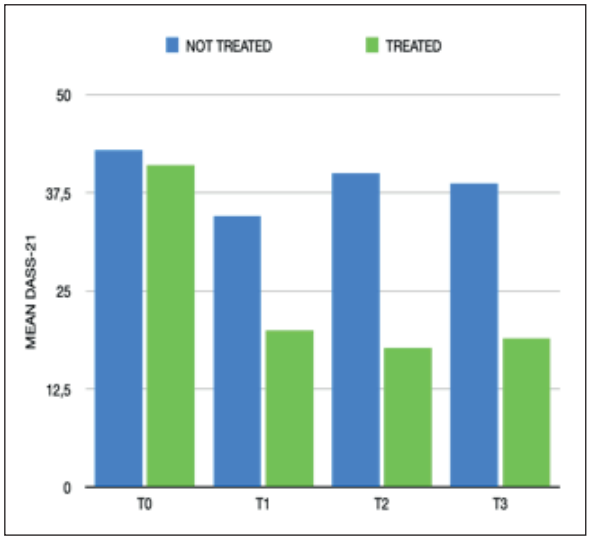

Figure 3A. Improvement of ASI score of treated patients during treatment. T1: 1 month, T2: 2 months, T3: 3 months.

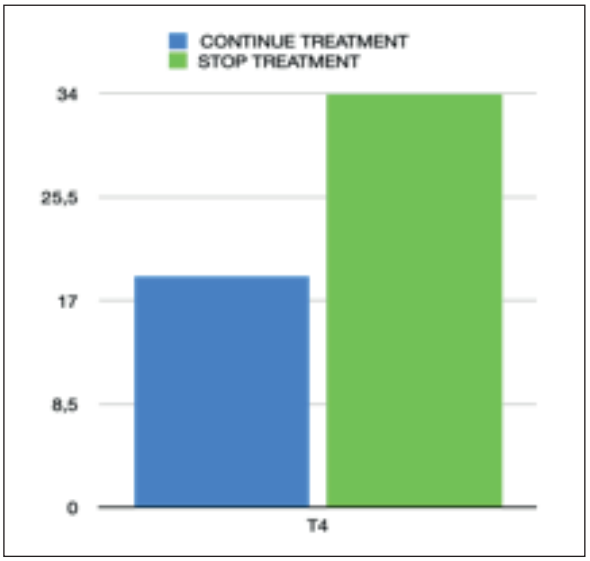

Figure 3B. Untreated patients return to a high ASI score, with a significant worsening of the mood. Treated patients maintain a low ASI score and a stable improvement of mood.

study opens the way to a new valid strategy to enhance the level of endogenous serotonin.

Conclusions

We observed a significant improvement of ASI on patients during HpPHIS therapy. Since serotonin is active on mood state and the presence of brain tissues receptors is known, we can forecast the possibility that an increase of serotonin levels in nasal cavities, due to HpPHIS, can lead to migration of serotonin along the olfactory fibers, to the brain, in the same way of NGF. We are studying an increased number of patients in a new study, aiming to better understand the pathway of serotonin from nasal cavities to the brain and its role in neurological diseases.

Conflict of Interest

All authors declare that they have no conflicts of interest.

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