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Circulating steroids negatively correlate with tinnitus

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ABSTRACT

While not a disease entity in itself; symptoms of tinnitus (from Latin tinnio – clink) accompany a number of diseases. Tinnitus prevalence increases with age, deteriorates one's quality of life, and may even result in suicidal behavior. Tinnitus develops in response to a variety of risk factors, otoxic substances, noise exposure, hearing disorders, and psychological alterations. Tinnitus is closely related to mood, depression, and psychological state. In the present study, we focused on alterations of the steroid metabolome and particularly neuroactive, neuroprotective, and immunomodulatory steroids in patients with tinnitus. The study group consisted of 28 patients without evidence of an organic cause of tinnitus as well as without associated diseases or the effect of ototoxic medications. All patients underwent a complete audiological assessment and laboratory tests including routine biochemical markers and quantification of circulating steroids using gas chromatography/mass spectrometry and immunoassays. To rule out a pathology in the cerebellopontine angle area, CT scan or MRI were performed. To diagnose stem lesions, evoked potentials were also measured. Pearson's correlations and multivariate regression were used to assess any links between tinnitus intensity and frequency on the one hand, and steroid levels on the other. Results indicated a significant and consistent negative correlation between tinnitus indices and intensity of adrenal steroidogenesis. The circulating steroid metabolome including hormones and neuroactive, neuroprotective, and immunomodulatory steroids negatively correlates with the degree of tinnitus due to hypothalamo-pituitary-adrenal axis malfunction. Our results may help explain the pathophysiology of tinnitus and improve its diagnosis. However, further studies are needed to verify our postulation.

1. Introduction

The aim of this work was to establish if patients with tinnitus without an organic cause develop alterations in the levels of bioactive steroids such as neuroactive, neuroprotective, and immunomodulatory steroids including their precursors and metabolites as the psychological status of each individual is clearly associated with the presence/absence of tinnitus, particularly in patients with depression [1–3].

The reported incidence of subjective tinnitus range from 8 to 15%. Population-based studies designed to assess hearing impairment in adult patients aged 48–92 years have reported a prevalence of 8.2% (start of study) with an incidence of 5.7% during 5-year follow-up [3–5]. Tinnitus prevalence has been shown to increase with age, whereas neurosteroid levels generally decline [6,7].

Neuroactive steroids (NASs) are produced in the central and peripheral nervous system (neurosteroids) as well as in the peripheral tissues; at the same time have the ability to accumulate in the nervous system [8]. However, NAS levels in the central nervous system (CNS) are only partially independent of NAS secretion from the steroidogenic glands and further peripheral tissues. Part of the CNS steroids is formed *de novo* from cholesterol while another part of them crosses the blood-brain barrier from peripheral tissues and may be further metabolized in CNS tissues (see review [9]). The group of enzymes important for the formation of critical neurosteroids includes various hydroxysteroid dehydrogenases, 5α-reductases, sulfatases, sulfotransferases, and 7α/β-hydroxylases [10–16].

As tinnitus is closely related to mood, depression, and the psychological state, we hypothesized that patients experiencing tinnitus might exhibit changes in the levels of neurosteroids. Animal experiments have demonstrated that, while the plasma concentrations of allopregnano-

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lone and allotetrahydrodeoxycorticosterone (THDOC) in acute stress increase, their levels are reduced during chronic stress [8,9]. Nevertheless, even though other neurosteroids have been studied relative to development of depression, conclusive evidence of a link between the pathophysiology of depression and neurosteroids is still lacking [17].

According to current knowledge, tinnitus can have one or more sources within the auditory pathway or in other parts of the CNS and neurotransmitters with their receptors playing an important role in tinnitus pathogenesis. The NASs have a significant ability to positively or negatively modulate the function of various neurotransmitter receptors [18]. The major excitatory neurotransmitter is glutamate. Its increased release at any point of the auditory pathway causes excitotoxicity and increased expression of N-methyl-D-aspartate (NMDA) receptors resulting in the development and perpetuation of tinnitus. Conversely, γ-amino butyric acid (GABA) is considered a major inhibitory neurotransmitter. NASs may also influence the functioning of a variety of further neurotransmitters [19].

2. Materials and methods

2.1. Subjects

The study group consisted of 12 adult women in (4 women in the follicular menstrual phase, 8 postmenopausal women) (55.2 ± 16.5 years of age, mean ± SD) and 16 adult men (52.5 ± 19.2 years of age, mean ± SD). The stage of the menstrual cycle in women was determined by circulating progesterone (< 7 nmol/L). The study group included patients without evidence of organic causes of tinnitus or a cause of non-vascular hearing impairment (Meniere's disease, acoustic trauma, etc.). The patients had no serious internal comorbidities that may affect results and were free of inflammation, cardiovascular diseases, diabetes mellitus, tumors, renal dysfunction, nor were they taking any ototoxic medication or medication affecting blood clotting. Their hearing loss was not greater than 40 dB.

All patients were examined by an internist and ENT specialist using otomicroscopy, epipharyngoscopy, audiometry with tinnitus masking, tympanometry, and questionnaires describing duration of the problem as well as tinnitus characteristics (high or low tones) were completed as well. Laboratory investigations included complete blood count, biochemistry, coagulation activity and quantification of circulating NASs as the NASs in brain tissues and cerebrospinal fluid are known to correlate with the corresponding values in the circulation [9,20–22]. To exclude a pathology in the cerebellopontine angle area, a CT scan or MRI imaging as well as X-ray of the cervical vertebrae were performed.

2.2. Statistical analysis

To estimate the associations between intensity and frequency of tinnitus on one hand and steroid metabolome on the other, we first used Spearman's correlation in respect of mostly non-Gaussian data distribution and, second, multivariate regression with a reduction of dimensionality known as the model of orthogonal projections to latent structures (OPLS) [23–26].

This more efficient method simultaneously evaluates the relationships between steroids and intensity of tinnitus, and is capable of coping with the problem of severe multicollinearity (high intercorrelations) in the matrix of predictors, while ordinary multiple regression fails to evaluate such data. The multicollinearity in OPLS is favorable as it enhances the predictivity of the model.

The variability shared between steroids and tinnitus intensity was separated in one predictive component and orthogonal components explaining the variability shared within the highly intercorrelated steroids independently of tinnitus intensity. Although necessary for OPLS model building, these orthogonal components were not interesting from the point of view of interpretation.

Orthogonal projections to latent structures identified the best predictors as well as the best combination of predictors to estimate tinnitus intensity. After standardization of the variables, the OPLS model can be expressed as follows:

$$\mathbf{X} = \mathbf{T}_p\mathbf{P}_p^T + \mathbf{T}_0\mathbf{P}_0^T + \mathbf{E} \quad (1)$$

$$\mathbf{Y} = \mathbf{T}_p\mathbf{P}_p^T + \mathbf{F} \quad (2)$$

where $\mathbf{X}$ is the matrix with predictors and subjects, $\mathbf{Y}$ is the matrix of dependent variables and subjects; $\mathbf{T}_p$ is the vector of component scores from the single predictive component and subjects extracted from $\mathbf{Y}$; $\mathbf{T}_o$ is the vector of component scores from the single orthogonal component and subjects extracted from $\mathbf{X}$; $\mathbf{P}_p$ is the vector of component loadings for the predictive component extracted from $\mathbf{Y}$; $\mathbf{P}_o$ is the vector of component loadings for the orthogonal component extracted from $\mathbf{X}$ and independent variables; and $\mathbf{E}$ and $\mathbf{F}$ are error terms.

The relevant predictors were chosen using variable importance of the projection (VIP) statistics. The statistical software used for OPLS analysis (SIMCA-P v.12.0 developed by Umetrics AB, Umeå, Sweden) enabled us to determine the number of relevant components, detect multivariate non-homogeneities, and test multivariate normal distribution and homoscedasticity (constant variance). The algorithm for obtaining the predictions is shown elsewhere [27].

2.3. Instruments and chromatographic conditions

A GCMS-TQ8040 system (Shimadzu, Kyoto, Japan) consisting of a gas chromatograph equipped with automatic flow control, an AOC-20s autosampler and a triple quadrupole detector with adjustable electron voltage of 10–195 V was utilized. The analysis was conducted in Q3-SIM mode. A capillary column with a medium polarity RESTEK Rxi column (diameter 0.25 mm, length 15 m, film thickness 0.1 μm) was used for analyses. Electron-impact ionization with electron voltage fixed at 70 V and emission current set to 160 μA was used for the measurements. The temperatures of the injection port, ion source, and interface were maintained at 220, 300, and 310 °C, respectively. Analyses were carried out in splitless mode with a constant linear velocity of the carrier gas (He) maintained at 60 cm/s. The septum purge flow was set at 3 mL/min. The samples were injected using high-pressure mode applied at 200 kPa and maintained for 1 min. The detector voltage was set at 1.4 kV.

2.4. Steroid analysis

In total, the levels of 46 analytes were quantified in the circulation of volunteers. These analytes included a group of liver function tests, 26 unconjugated steroids and 20 steroid conjugates. The steroid metabolome in the maternal circulation included the levels of C21 Δ^5 steroids, C19 Δ^5 steroids, C21 Δ^4 steroids, C19 Δ^4 steroids, estrogens, C21 5α/β-reduced steroids, and C19 5α/β-reduced steroids. Most of the steroids were measured by GC–MS using our previously published method (for details, see [28–30]); however, 17-hydroxyprogesterone and cortisol were quantified by RIA kits (Immunotech, Marseille, France) (for the steroids and steroid abbreviations, see Table 1).

2.5. GC–MS analysis

Samples for GC–MS analysis were prepared as follows: unconjugated steroids were extracted from 1 mL of serum fluid with diethyl-ether (3 mL). The diethyl-ether extract was dried in a block heater at 37 °C. The lipids in the dry residue of the diethyl-ether extract were separated by partitioning between a mixture of methanol–water 4:1 (1 mL) and pentane (1 mL). The pentane phase was discarded and the polar phase was dried in a vacuum centrifuge at 60 °C (2 h). The dry residue from the polar phase was derivatized first with methoxylamine hydrochloride solution in pyridine (2%) on oxo-groups (60 °C, 1 h). The