The role of the serotonin and CGRP in migraine: Genetic and neurochemical studies

Ph. D. Theses

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1 Introduction

Our understanding of the pathophysiological mechanisms of migraine remains poor despite the availability of clinically effective drugs and many years of research (6). In the 1940s two independent theories of headache aetiology were suggested, but these could only give a partial explanation to the cause and symptoms of migraine. The vascular hypothesis proposed by Wolff and his colleagues was based upon the observation that direct stimulation of the large cerebral vessels produces a pain similar to migraine pain, while during a migraine attack the diameter of these blood-vessels change. The neurogenic migraine theory, originally proposed by Leao and his research team, sets out of the fact that the alteration of blood flow develops as a consequence of cortical spreading depression which traverses the cerebral cortex at a velocity of 2-5 mm/min. Thanks to improvements of our biochemical knowledge, it became evident that serotonin plays a pivotal role in the development of migraine. This was supported by several clinical observations which pointed to changes in the serotonergic metabolism during the migraine attack, in migraineurs (1). In addition, the agents that release serotonin, or some particular serotonergic receptor agonists provoke migraine attacks (2). The importance of serotonin is also supported by the fact that most of the anti-migraine drugs act through the serotonergic system (3). The neurogenic dural inflammation theory of migraine which was developed by Moskowitz and Fozard, tried to combine the latest neurochemical results and the theories mentioned above (2, 5).

Several genetic epidemiological and segregation studies confirm that the most frequently appearing variants of the migraine are polygenic multifactorial diseases (4). Great improvements of the molecular genetic methods resulted in a fundamental change in the research fields. In present-day migraine research, the genetical background of biochemical parameters needs to be investigated.
2 Objectives

The present dissertation deals with still unclear aspects of the relationship of the serotonergic system and migraine. Previously it has been suggested that serotonin does not play a causative role in migraine, but is taking part in complicated pain controlling processes. On the other hand – based on the results of the experimental, mainly animal studies – it seems that the CGRP (calcitonin gene-related peptide) plays an essential role in the development of the migraine pain. Unfortunately, extremely little human evidence is available and the results based on the animal migraine-models are questionable at several points. We also lack human clinical data of the interaction of serotonin and the CGRP. Another major problem is that we still do not understand the real mechanism of action of the anti-migraine drugs acting through the serotonergic system. Considering all these facts, I will try to find answers to the following questions:

1) Is there any difference in the serotonergic metabolism between migraine patients and control persons in our study population?

2) Do the certain polymorphisms in the serotonin transporter gene and the 5-HT$_{2A}$ receptor gene play a role in the development of the migraine?
   a) Do the allele and the genotype frequencies of migraine patients vary in comparison to the control population?
   b) Is there any association between the platelet serotonin concentration and the functional 5-HTTLPR polymorphism of the 5-HT transporter gene?
   c) Can the functional 5-HTTLPR polymorphism of the serotonin transporter gene be a risk factor for migraine, by itself?

3) Can the role of the serotonin and the CGRP be verified in the nitroglycerin induced migraine attack which is considered the human migraine model?
   a) Are the migraine patients more sensitive to the NO donor nitroglycerin?
   b) What kinds of neurochemical factors predispose to the development of the NO induced migraine?
   c) What kinds of neurochemical changes occur during the migraine attack?
d) What kind of relationship do the neurochemical changes have with the migraine pain?

e) What kind of neurochemical changes does the sumatriptan therapy used in the migraine treatment cause?

3 Methods

3.1 Study subjects

Unrelated migraine patients (migraine without aura and migraine with typical aura), selected from the National Institute of Psychiatry and Neurology, Headache Clinics of Sport Hospital and Neurological Department of the Health Service of Zuglo, Budapest, Hungary, were included in the studies. The healthy controls were students and hospital staff. All individuals were female and have Caucasian origin. A detailed medical history was taken from each subject, especially concerning headache and other neuro-psychiatric disorders. The subjects underwent complete physical, neurological and psychological examinations, as well as laboratory tests, before participating in the projects. The headache diagnoses were obtained according to the criteria of the International Headache Society. In our studies we compared the data of the headache patients to that of the healthy controls. The study protocols were approved by the Ethics Committee of the National Institute of Psychiatry and Neurology, for experimentation on humans, and every subject gave written informed consent before participating in the research.

3.2 Measured parameters

- The serotonin concentrations were determined from platelet-rich-plasma by high-pressure liquid chromatography (HPLC), coupled with electrochemical detection.
- The estradiol and progesterone concentrations were measured by standard radioimmunoassay (RIA) methods.
- The serotonin transporter gene 5-HTTLPR polymorphism was genotyped by PCR.
- The 102T/C polymorphism in the 5-HT2A receptor gene was determined by PCR and digestion with restriction enzyme Msp.I.
- Plasma CGRP concentrations were measured by means of a specific and sensitive radioimmunoassay (RIA) method.
- Self-monitoring daily headache diaries and daily medication records were used to evaluate the headache frequencies, the quality of headaches and the treatment outcome.
- The headache intensity was scored on a verbal scale that measured from 0-10.
- The quality of the headaches and the effectiveness of the treatment was recorded with the standardized questionnaires.

3.3 Statistical analysis

One-way, two-way and repeated measure analyses of variance (ANOVA) with post hoc comparisons (Newman-Keuls) were applied for analysing the received data. Friedman’s nonparametric ANOVA and the Mann-Whitney U test were used to test the nonparametric results. Significance levels were adjusted for multiple comparisons using a Bonferroni correction. The chi-squared test was applied to determine the statistical division of the participants, alleles and genotypes in the different groups. Correlations were quantified using the Spearman rank order correlation coefficient and Pearson product moment correlation test. The logistic regression model was used to test the interaction between the two genes. Odds ratios and 95% confidence limits were calculated by standard methods.

4 Results

The aim of my studies was to get more acquainted with the relationship of the serotonergic system and migraine, and to examine the neurochemical changes during a migraine attack. My results could be summarized as follows:
- The platelet serotonin concentrations are significantly lower in migraine patients without aura in headache free period compared to controls carefully matched by sex, age and phase of menstrual cycle.

- A locus at or near the serotonin transporter gene is a possible risk factor for migraine, because the frequency of the 5-HTTLPR polymorphism’s S allele is significantly higher in migraineurs than in controls. However, the lower serotonin concentration in migraine patients is not a consequence of the differing allele frequency of the 5-HTTLPR polymorphism, so the two risk factors can occur separately. The “stress sensitive” S allele may express the greater susceptibility to migraine by increasing the anxiety related traits.

- The 5-HT2A receptor gene has no effect on the development of migraine.

- The lower platelet serotonin concentration and higher basal CGRP concentration in the headache free period are risk factors for migraine that express greater susceptibility to develop both spontaneous and NO-induced migraine attack.

- The plasma CGRP concentration is a dynamically changing trait marker – both in timing and severity – for the migraine-induced pain which suggests a possible causative role of CGRP in migraine.

- The plasma CGRP concentrations failed to change during the immediate, mild headache induced by nitroglycerin.

- In the nitroglycerin-induced migraine model the serotonin release from platelets does not causally relate to the migraine attack, on the contrary, it may prevent or alleviate the migraine pain, presumably by the activation of the 5-HT1B/1D receptors.

- The 5-HT1B/1D receptor agonist sumatriptan decreases the plasma CGRP concentration in parallel with the headache intensity, most probably this plays a role in the anti-migraine effect.
5 Summary

The aim of the present study was to evaluate the role of the CGRP and the serotonergic system in the development of migraine. With this approach, I investigated the genetic and neurochemical risk factors of migraine, and studied how these neurochemical parameters change during a migraine attack.

Our data support that the platelet serotonin concentrations are significantly lower in migraine patients without aura in the headache free period compared to controls. By examining the genetic background of the association between platelet serotonin concentrations and migraine we demonstrated that the frequency of the S allele of the serotonin transporter gene 5-HTTLPR functional polymorphism is significantly higher in migraineurs than in controls. However, the lower platelet serotonin concentration in migraine patients is not a consequence of the differing allele frequency of the 5-HTTLPR polymorphism. The higher anxiety level of the migraineurs suggested that the “stress sensitive” S allele of the serotonin transporter gene may express a greater susceptibility to migraine, by itself. Furthermore, we supported that the 5-HT2A receptor gene has no effect on the development of migraine.

The NO donor nitroglycerin induced migraine attack is the most commonly used human migraine model. Our results support that the lower platelet serotonin concentration and higher basal CGRP concentration in a headache free period are risk factors for migraine that express greater susceptibility to develop both spontaneous and NO-induced migraine attacks. According to our results the plasma CGRP concentration is a dynamically changing trait marker for the migraine-induced pain that increases with the pain. On the other hand, the 5-HT1B/1D receptor agonist sumitraptan causes a decrease in the plasma CGRP concentration in parallel with the ease of the migraine attack. Furthermore, the plasma CGRP concentrations failed to change during the immediate, non migraine-type, mild headache induced by nitroglycerin. With these results we could present human evidence that the CGRP plays a causative role in the development of the migraine.

Finally we can state that in the nitroglycerin-induced migraine model the serotonin release from platelets does not causally relate to the migraine attack, on the contrary, it may prevent or alleviate the migraine pain.
6 References


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8 The dissertation is based on the following publications:

8.1 Journal articles:


8.2 Citable abstracts


9 Other publications, journal articles, oral and poster presentations


10. Juhász, G. Receptorok genetikai polimorfigmusának jelentősége a klinikai gyakorlatban. (Significance of the receptor’s genetic polymorphisms in clinical