Molecular and clinical study of the vestibular function

Thesis

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INTRODUCTION

The vestibular compensation after unilateral peripheral vestibular loss is a modell of the plasticity of the brain. If we want to investigate it, we should do it on molecular and on clinical level as well.

After unilateral labirynthectomy (UL) a statical deficit (ocular nystagmus and postural syndrome) can be observed. After 3 days the main symptoms caused by the lesion are ceased. This process called *vestibular compensation*.

During this process the deafferentated vestibular neurons reach their rest activity. The peripheral vestibular receptors are not capable for the regeneration, so this compensation must be because of the plasticity of the brain.

The peripheral vestibular deficit is a good model to investigate the vestibular compensation, because:

- the peripheral lesion is easy to examine,
- the anatomy, physiology and function of this region is well-investigated.

AIMS

In our studies we approched to the vestibular system and vestibular compensation from two sides: molecular and clinical.

In the **molecular part** we investigated the intrinsic membrane properties of the central vestibular neuron and the facial motoneuron with in situ hybridization technique. We registrated the quantitative change of the voltage-dependent sodium and Ca-dependent potassium channels in different postlesional days: 1, 3, 8 and 30.

We investigated the facial motoneurons because of two reasons:

- the surgical technique of labyrinthectomy gives the opportunity for the section of the nerve,
- the examination of the axotomized facial neurons is another opportunity for the investigation of the central plasticity.

The other part of our study was the **clinical part**.

In the neuro-otological examinations existed no possibility to check the function of the otolith apparatus. We registrated the vestibular-evoked myogenic potentials (VEMP) to assess the function of sacculus and the vestibulo-colic pathways.

In our study we examined patients suffering from unilateral vestibular schwannoma (1. study) and multiple sclerosis (2. study). We tried to understand how useful is the VEMP in the vestibular diagnostic, and could it give more information for us in central and peripheral vestibular disorders.

METHODS

Molecular studies

Surgical procedures: labirynthectomy and facial nerve transsection

In the first study we used 51, in the other one we used 30 grown, male, pigmented, 200-300 g weight Long-Evans rats. The animals have been divided into 2 groups: an experimental group (n=42, and 21) and a control group (in both studies n=9).

The experimental group was anesthetised with halothane and unilateral labyrinthectomy and facial nerve transsection under surgical microscope was performed.

In situ hybridization and autoradiography

Animals

The in situ hybridization was performed in both experimental and control groups. The rats were divided into the following groups:

- A: 12 rats, the investigation of the mRNA of Na α1 and α3 channels
- B: 30 rats, the investigation of the mRNA of SK-1, 2 and 3 channels
- C: 21 rats, the investigation of the mRNA of the Na β1, β2 and β channels

Every experimental group was subdivided into 3 groups and the animals were killed on different postoperative days.

The steps of the process were the following:

- *Tissue preparation*
- *Oligonucleotide probes*

For Na α- and SK-channels we used 2, for the Na β-channels one antisense oligonucleotide probe.

- *In situ hibridization and autoradiography*
- *Autoradiogram analysis*
- *Statistical analysis*

We used non-parametric tests (Kruskal-Wallis, Wilcoxon and Mann-Whitney test, Statistica, Statsoft Inc.). The level of significance was $P<0,05$.

Clinical studies

Vestibular schwannoma

We examined 95 healthy persons (63 women and 32 men, average age: 41,6 years of age, age limits: 14-78 years of age), who gave the control group, and 170 patients suffering from unilateral vestibular schwannoma (94 women and 76 men, average age: 51,4, age limits: 16-81 years of age). We performed subjective audiometry, complete neuro-otological examination and by schwannoma patients an MRI scan.

Vestibular-evoked myogenic potentials

By every patients we performed click-and STB-evoked VEMP-registration. We registered the P13-N23 latency (in ms) and peak-to-peak amplitude (microV). By patients with schwannoma we calculated the VEMP-assymetry (EPR= evoked potential ratio).

Caloric test

We used the Dix-Hallpike-Fitzgerald method with 30 and 44 Celsius water. To assess tha canal paresis we used the Jonkees formula.

Audiometry

We performed subjective audiometry by every subject.

Statistical analysis

We used analysis of variance (ANOVA), the level of significance was: $P< 0,05$.

Multiple sclerosis

We examined 30 healty persons (11 women and 19 men, average age: 45 years of age, age limits: 21-75 years of age), who gave the control group, and 30 patients suffering from multiple sclerosis (20 women and 10 men, average age: 43,4, age limits: 27-60 years of age).

The subjects suffering from the following pathologies

were not included in the study:

- conductive hearing loss,
- peripheral vestibular damage,
- pathology of the sternocleido-mastoid (SCM) muscle.

By every patients we performed subjective audiometry, complete ENT, neurological and neuro-otological examination and by MS patients an MRI scan.

By every patients we performed click-evoked VEMP-registration with Cadwell Sierra EMG/EP machine (Cadwell Laboratories, Inc., Kennewick, WA, USA). We registered the P13-N23 latency (in ms) and peak-to-peak amplitude (microV). We also calculated the P13 interside difference.

For statistical analysis we used Mann-Whitney- test and chi-squared test, the level of significance was: $P< 0,05$.

RESULTS AND CONCLUSIONS

In our studies we approched the vestibular system and vestibular compensation from two sides: molecular and clinical.

In our ***molecular studies*** we found, that unilateral removal of one labyrinth does not affect the abundance of mRNAs coding for the subunits SK1, SK2 and SK3 of the calcium-dependent potassium channels, and for the subunits Na αI and Na αIII and Na β1-3 sodium channels in the deafferented vestibular neurons during the first month following the lesion. Our data therefore do not support the idea of a widespread dynamic modulation of the expression of ion channels by vestibular activity in the vestibular system of adult rats.

In contrast, a strong modulation of the expression of the genes for sodium- and calcium-dependent potassium channels was observed in the axotomized facial motoneurons. The changes in the abundance of these conductance mRNAs may

explain the long-term hyperexcitability previously described in these cells following axotomy.

In our ***clinical studies*** we used VEMP (vestibular evoked myogenic potential), which is now a well-established test to explore the sacculo-collic pathways in human. Loud monaural clicks evoke an initial inhibitory potential in tonically contracted ipsilateral SCM. This potential is responsible for the early waves. P13-N23 and has been demonstrated to be of saccular origin.

We investigated 170 patients suffering from unilateral vestibular schwannoma:

- 78.8% exhibited a dysfunction of the sacculo-collic pathways as evidenced by the absence of or abnormally low VEMP response on the affected side to clicks and/or STB.

- No response was induced by clicks in 69.4% of the 170 patients, but a normal or a low saccular response was evoked by 500 Hz STB in 23.5% of this subgroup. Thus, STB are a

more effective than clicks as a stimulus for the sacculo-collic pathways.

- The P13 latency to clicks or STB was prolonged in only 18/170 patients (10.5%) whereas it was normal in the other 152/170 patients (89.5%).

- The absence of strong, systematic correlation between the findings of caloric tests, VEMPs and audiometric tests indicates that acoustic neuroma may selectively affect the saccular, auditory or horizontal canalar nerve.

We confirmed here previous data in smaller series showing that the VEMP showed abnormal findings in about 80% of acoustic neuroma patients. In addition, we showed for the first time in a large number of patients that the response to the VEMP test induced by high level clicks and to 500Hz STB may be different. In particular, a normal or reduced response could be evoked by STB even in the absence of clicks-VEMP on the affected side whereas the reverse was not true. We suggest thus that clicks are more appropriate than STB to appreciate a slight saccular dysfunction and consequently to detect small tumors of the vestibular nerve. The acoustic

neuroma could cause lesion separately on the saccular, auditory or horizontal canalar nerve as assessed by the absence of correlation between caloric tests, VEMPs and audiometric tests.

We also studied patients suffering from multiple sclerosis, where we found 40 % of the patients with abnormal VEMP. We found, that VEMP abnormalities show the strongest correlation with demyelinative MRI lesions in the brainstem and a weaker correlation with the duration of the disease. The clinical signs of vestibular dysfunction don't seem to affect the chances of obtaining abnormal results. Although the sensitivity of VEMP in detecting abnormality in MS patients is relatively low, as compared to other evoked potential modalities, its significance lies in that it is an easily and quickly performed electrophysiological method assessing the function of central vestibular pathways. This relatively newly introduced method provides a further tool to the clinician.

LIST OF PUBLICATIONS

1. Patko T, Vidal PP, Vibert N, Tran Ba Huy P, de Waele C. (2003) Vestibular evoked myogenic potentials in patients suffering from an unilateral acoustic neuroma: a study of 170 patients. Clin Neurophysiol,114: 1344-1350. **IF: 2,485**

2. Patko T, Vassias I, Vidal PP, De Waele C. (2003) Modulation of the voltage-gated sodium- and calcium-dependent potassium channels in rat vestibular and facial nuclei after unilateral labyrinthectomy and facial nerve transsection: an in situ hybridization study. Neuroscience, 117: 265-280. **IF: 3,601**

3. T Patkó, M Simó, Z Arányi. (2007) Vestibular click-evoked myogenic potentials: sensitivity and factors determining abnormality in patients with multiple sclerosis Mult Scler, 13: 193-198. **IF: 2,832**

4. Vassias I, Patko T, Vidal PP, de Waele C. (2003) Modulation of the beta1-3 voltage-gated sodium channels in rat vestibular and facial nuclei after unilateral labyrinthectomy and facial nerve section: an in situ hybridization study. Brain Res Mol Brain Res, 120:73-78. **IF: 2,107**

LIST OF PRESENTATIONS

2002. Marseille, France

2003. Paris, Congress of the Bárány Society

2006. Debrecen, National ENT Congress

OTHER PUBLICATIONS ON VESTIBULAR THEME

1. Mbongo F, Patko T, Vidal PP, Vibert N, Tran Ba Huy P, de Waele C. (2005) Postural control in patients with unilateral vestibular lesions is more impaired in the roll than in the pitch plane: a static and dynamic posturography study. Audiol Neurootol, 10: 291-302. **IF: 2,108**