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Neuromuscular transmission in migraine patients with prolonged aura

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Abstract

P/Q Ca^{2+}channels are genetically abnormal in most cases of familial hemiplegic migraine (FHM) and may be involved in other types of migraine. They are also found at the neuromuscular junctions, where they control stimulation-induced acetylcholine release. Prolonged aura is a very frequent clinical feature in FHM patients. The objective of this study was thus to explore neuromuscular transmission in migraine with typical and prolonged aura patients. We performed single fiber electromyography (SFEMG) in such patients and compared them to a group of healthy volunteers. Results were expressed as mean jitter (MCD) and percentage of single endplate abnormalities. Mean MCD was on average comparable in controls and migraineurs. By contrast, single endplate abnormalities were only found in patients ($p<0.01$), especially in those with prolonged aura ($p<0.001$). These results suggest subtle impairment of neuromuscular transmission in a subgroup of migraineurs characterized by prolonged aura, which might be due to dysfunctioning P/Q Ca^{2+}-channels.

Key words : familial hemiplegic migraine ; migraine ; neuromuscular transmission ; P/Q Ca^{2+} channels ; prolonged aura ; single fiber electromyography

Introduction

There is ample evidence for genetic involvement in migraine, mainly coming from segregation analyses (Russell and Olesen, 1993 ; Russell *et al*., 1996) and population studies (Gervil *et al*., 1999, Kallela *et al*., 1999). Mutations in the α_{1A}-subunit gene of the neuronal voltage-dependent P/Q-type Ca^{2+} channel (CACNA1A gene on chromosome 19p13) have been found in familial hemiplegic migraine (FHM) as well as in episodic ataxia type 2 (EA-2) and spino-cerebellar ataxia type 6 (SCA6) (Ophoff *et al*., 1996 ; Jodice *et al*., 1997 ; Zhuchenko *et al*., 1997).

Sib-pair (May *et a*l., 1995) and linkage (Nyholt *et al*., 1998) analyses have provided indirect evidence that mutations in CACNA1A may also be accountable for more frequent forms of migraine, especially for migraine with aura. P/Q Ca^{2+} channels are widely distributed in the nervous system, particularly in the cerebral cortex (Mintz *et al*., 1992), in cerebellum (Stea *et al*., 1994 ; Randall *et al*., 1995) and in presynaptic motor axons at the neuromuscular junctions, where they are responsible for stimulation-induced acetylcholine release (Sugiura *et al*., 1995). A dysfunction of these ion channels may therefore modify a range of neuronal functions. Abnormal electrophysiological patterns, found interictally in migraineurs, such as deficient habituation of pattern-reversal visual evoked potentials (Schoenen *et al*., 1995 ; Afra *et al*., 1998) and strong intensity dependence of auditory cortical evoked potentials (Wang *et al*., 1996) , have a strong genetic component (Sandor *et al*., 1999). Subclinical cerebellar signs were recently found in migraine patients with a 3D optoelectronic video analysis of free-reaching movements (Sándor *et al*., 1998). In previous studies (Ambrosini *et al*., 1999 ; Ambrosini *et al*., 2001), we used single fiber electromyography, the most sensitive *in vivo* method, to investigate neuromuscular transmission in migraine patients. We found subclinical impairment of neuromuscular transmission in a subgroup of migraineurs, who were characterized by clinical features such as unilateral sensorimotor symptoms, aphasia and vertigo, and prolonged aura, associated or not with visual aura symptoms. None of them fulfilled the IHS criteria for FHM (Headache Classification Committee of the International Headache Society, 1988). As these symptoms are also hallmarks of human P/Q Ca^{2+} channelopathies such as FHM and episodic ataxia type 2 (Terwindt *et al*., 1998), we hypothesized that the abnormalities of neuromuscular transmission detected by SFEMG might reflect dysfunctioning presynaptic P/Q Ca^{2+}-channels on motor axons. As prolonged aura exceeding 60 minutes is a frequent finding in FHM (Ducros *et al*., 2001), the purpose of the present study was to extend our electrophysiological investigations of the neuromuscular junction in migraine with aura, focusing attention on the duration of the aura. We compared therefore two groups of patients suffering from migraine with aura, one with typical and and one with prolonged aura, in order to further explore and eventually confirm our

preliminary observation of a positive correlation between the duration of the aura and performance at the neuromuscular.

Subjects and methods

Subjects. — Thirty-seven migraine patients took part in the study. Twenty-four were affected by migraine with typical aura (MTA ; IHS code : 1.2.1) and 13 by migraine with prolonged aura (MPA ; IHS code 1.2.2). Detailed records of clinical aura features additional to the visual disturbances were kept for each patient. The symptoms taken into consideration were sensory and motor troubles, aphasia and equilibrium disorders such as vertigo or ataxia.

We also selected a proper control group of subjects (healthy volunteers : HV; n = 16) for having no known personal or familial history of recurrent headaches nor any other neurological disorder.

None of controls or migraineurs were taking drugs on a regular basis, nor had they taken any drug within three days before the recordings.

Experimental Procedures. — Migraineurs were examined interictally at an interval of at least three days from an attack by one of us (A.A.) who was blinded to the detailed clinical history. A *Viking* IV device *(Nicolet® Biomedical - Madison, Wisconsin, USA)* was used for stimulation single fiber electromyographical recordings (Stalberg and Trontelj, 1997). Single muscle fiber electrode activity was recorded with 25 mm long single fibre needles *(ref : 16829 Medelec™ Neurodiagnostic Accessories, Witney, Oxfordshire, UK)* and the motor nerve was stimulated with Nicolet™ teflon-insulated monopolar needles. We stimulated a motor branch of the radial nerve and assessed the variability in latency, i.e., the jitter, of single endplate action potentials in *m. extensor digitorum communis* (EDC) of the right arm. Stimulation rate was 10 Hz and on average we recorded 18 EDC muscle fibers per patient. Results were expressed as the ‘mean value of consecutive differences’ (MCD) of successive interpotential intervals, as usual in SFEMG studies. According to published normative data (Sanders and Stalberg, 1996), mean MCD may not exceed 25 µsec and no more than 10% of fibers are allowed to have an MCD superior to 40 µsec; in more severe impairments of neuromuscular transmission, nerve impulses may fail to elicit an action potential, producing intermittent impulse blocking. As we tested a proper control group, in order to avoid inclusion of subjects affected by, or at risk of migraine, who occasionally may have been included in formerly published normative data, we considered mean MCD values higher than the mean MCD value in these controls plus 2SD as “mean MCD abnormalities”and the presence of fibers with increased jitter and/or intermittent impulse blocking as “single endplate abnormalities”.

Statistical analysis. — Results were expressed as means and standard deviations (SD) for quantitative variables and as proportions for binary data. Proportions were compared by the classical chi-square test for contingency tables. Normality of mean values has been assessed by the Shapiro-Wilk’s W test and they have been compared by the t-test for independent samples for the group analysis and by the one-way ANOVA post-hoc comparison (LSD test) for between group analysis. All results were considered to be significant at $p<0.05$. Statistical calculations were carried out using the STATISTICA program (version 5.0 for Windows).

Results

Healthy volunteers and migraineurs had a similar sex and age distribution. Migraine subgroups did not differ with respect to age and mean monthly frequency of attacks, even though there were less women suffering from MTA (*p=0.012*) (Table 1). The prevalence of additional aura features besides visual symptoms was not significantly different

Table 1

Gender ratio, mean age and mean monthly attack frequency in the total group in the various groups of subjects

Groups	*mean age (years)*	*Gender (F/M)*	*mean monthly attack frequency (attacks/month)*
HV (n=16)	34.7 ± 11.2	10/6	0
MTA (n=24)	34.1 ± 13.5	10/14	2.8 ± 2.6
MPA (n=13)	34.9 ± 10.8	11/2	2.6 ± 2.2
All migraineurs (n=37)	34.4 ± 12.4	21/16	2.7 ± 2.4

HV : healthy volunteers. MTA : migraine with typical aura. MPA : migraine with prolonged aura.

Table 2

Mean MCD values and percentage of subjects with mean MCD and single endplate abnormalities for each group of subjects

Groups	*Average mean MCD (µsec)*	*Subjects with abnormal mean MCD*	*Subjects with single endplate abnormalities*
HV (n=16)	17.1 ± 2.6	1 (6.25%)	0
MTA (n=24)	17.6 ± 3.9	4 (16.7%)	5 (20.8%)
MPA (n=13)	20.6 ± 4.1**	4 (30.8%)	7 (53.8 %) **
All migraineurs (n=37)	18.7 ± 4.2	8 (21.6%)	12 (32.4 %) *

HV : healthy volunteers. MTA : migraine with typical aura. MPA : migraine with prolonged aura. MCD : mean value of consecutive differences of stimulus- single fiber potential intervals. * : significant versus HV. ** : significant versus HV and MTA.

between patients with prolonged aura (84.6%) and those with typical aura (62.5%).

Mean MCD was comparable in controls and migraineurs, 17.09 ± 2.65 μsec and 18.66 ± 4.18 μsec respectively (*p=0.173*). By contrast, mean MCD was higher in migraineurs with prolonged aura (20.63 ± 4.13 μsec) if compared with migraineurs with typical aura (17.60 ± 3.88 μsec, *p=0.019*) and healthy volunteers (*p=0.012)* (Table 2).

Mean MCD abnormalities, as defined in the Methods section, were observed in one out of 16 healthy volunteers and in 8 out of 37 patients, a non-significant difference. By contrast, single endplate abnormalities were absent in all volunteers and present in 12 patients (*p = 0.0096*). Nine of them had abnormal single endplate MCD values without impulse blocking (on average 8.02 ± 2.56 % of explored endplates; average mean MCD in these endplates : 45.77 ± 4.15 μsec) ; one had only intermittent impulse blocking (10.00 % of explored endplates ; 16.6 % of impulse blockings in these endplates) and two patients had both (5.8% and 15% of explored endplates with abnormal MCD ; mean MCD in these endplates : 58 μsec and 43.3 μsec ; 17.6% and 15% of explored endplates with intermittent impulse blocking ; 8.3 % and 6.5 % of impulse blockings in these endplates).
Single endplate abnormalities were not evenly distributed in the group of patients. They were more prevalent in MPA patients compared to healthy volunteers (p=0.0008) and to MTA patients (p=0.04) (Table 2).

Conclusions

In our previous SFEMG studies, we found evidence for subclinical impairment of neuromuscular transmission in some patients suffering from migraine with aura (Ambrosini *et al*., 1999, Ambrosini *et al*., 2001). Many of them were characterized by peculiar clinical features during the aura, such as unilateral motor or sensory deficits or both, language impairment, equilibrium disorders and prolonged duration of the aura. In the present study, we focused on the temporal aspects of the neurological aura and confirm that subclinical impairment of neuromuscular transmission is more prevalent in patients who regularly experience prolonged auras than in those who have a typical aura lasting no more than one hour.

Prolonged aura is rather frequent in familial hemiplegic migraine. The average duration of aura in these patients is 1-2 hours, even though it may last less than an hour. Moreover, about one-third of FHM patients describe atypical attacks characterized by prolonged aura lasting up to five days (Ducros and Campbell, 2000 ; Ducros *et al.*, 2001). The frequency of atypical and severe attacks is higher in the group of FHM families with cerebellar signs (Ducros *et al.*, 2001). As a large number of FHM families have mutations in the a_{1A}-subunit gene of the P/Q-type Ca^{2+} channel (CACNA1A) on chromosome 19p13, the clinical feature of prolonged aura might be related to dysfunctioning P/Q channels. The mild SFEMG abnormalities that we found in some migraineurs might thus be an index of the suboptimal performance of these channels at presynaptic motor axons. Interestingly, in tottering mice which present mutations in the CACNA1A gene, *in vitro* electrophysiology of the neuromuscular junction has revealed that high rate-evoked acetylcholine release is depressed, suggesting a reduced safety factor of neuromuscular transmission (Plomp *et al*., 2000).

Although data on SFEMG recordings are not yet available in FHM, patients with EA-2 and CACNA1A mutations had pronounced SFEMG abnormalities in a recent preliminary report (Jen *et al*., 2000).

If abnormal SFEMG findings reflect genetically abnormal α_{1A}-subunits, certain patients suffering from migraine with aura, especially those with prolonged auras, might share the same genetic background as familial hemiplegic migraine and express a milder form of this channelopathy. As indicated by Terwindt *et al*. (1998), some members of FHM families carrying the I1811L FHM mutation have migraine with aura but no attacks of hemiplegic migraine. Sib-pair analyses, performed in affected sibs of migraine without and with aura families, have shown an increase of shared marker alleles of locus D19S394, which is tightly linked to the CACNA1A gene (May *et al*., 1995). Conversely, linkage studies in migraine families have produced conflicting results. On the one hand, Hovatta *et al.* (1994), using two-point and multipoint parametric linkage analysis, did not find any evidence for involvement of the FHM locus in typical migraine. On the other hand, Nyholt *et al*. (1998), using parametric and nonparametric linkage analyses, suggested the implication of the CACNA1A gene in one out of four families investigated, underscoring the genetic heterogeneity of familial typical migraine. How the P/Q Ca^{2+} channel dysfunction may be involved in the pathogenesis of migraine, is still debated.

If ongoing genetic analyses can prove that migraine patients with SFEMG impairments have genetic abnormalities such as mutations or polymorphisms in the CACNA1A gene, single fiber electromyography could be useful to select patients for genetic analyses and hopefully for novel adequate therapeutic approaches.

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