The onset of the migraine attack

Oosterhout, W.P.J. van

Part IV.

Summary

Chapter 11.

Conclusions, general discussion and future perspectives

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For this thesis, clinical investigations on the premonitory phase and early phase of both the spontaneous and triggered migraine attack were performed. In the first part, we studied clinical aspects and modulators of migraine attacks. In the second part, we used magnetic resonance imaging techniques to study metabolic and perfusion changes in the hypothalamus during the premonitory phase and early headache phase of both spontaneous and glyceryl trinitrate triggered migraine attacks. Lastly, several biochemical modulators and triggers of migraine attack onset were investigated.

Part I: Clinical aspects and modulators

Migraine is a clinically heterogeneous headache disorder characterized by recurrent, disabling attacks of severe and often unilateral headaches, accompanied by symptoms of photophobia, phonophobia, nausea and vomiting [1]. In one third of patients, transient neurological aura symptoms precede the headache [2]. It is pivotal for both epidemiological and clinical experimental studies to clearly distinguish between migraine with and without aura.

Diagnosing migraine aura reliably using a questionnaire

In epidemiological and genetic studies, but also in clinical experimental settings, it is important to be able to clearly distinguish between migraine patients with and without aura. These aura symptoms are most often visual, although sensory, motor or speech related symptoms can occur as well. Diagnosing patients can best be done using an interview. However, when the inclusion of large numbers of patients is necessary, accurate and reliable questionnaires are a well-desired tool. In **chapter 2** we assessed the validity of a self-administered, web-based migraine questionnaire in the setting of the well-defined Leiden University Migraine Neuro-Analysis (LUMINA) cohort. Computer-aided diagnoses were checked by semi-structured telephone-interviews in 1,067 patients. We compared a subset of seven questions with the full algorithm based on the ICHD-2 criteria [3, 4] and found that this subset provided a higher sensitivity (86% vs 45%), slightly lower specificity (75% vs 95%) and similar positive predictive value (86% vs 88%) in assessing aura. The study was the first to corroborate that an online questionnaire can accurately and reliably diagnose aura and enables detection of more aura cases with a low false-positive rate for the use in future epidemiological and genetic research.

Chronotypes and restless legs; underlining the link between migraine and the hypothalamus

Since migraine attacks also show seasonal and circadian periodicity [30-34], it is suggested that chronobiological, probably hypothalamic-mediated mechanisms play a role in the triggering and initiation of migraine attacks as well. In **chapter 3** migraine status was studied in relation to chronotype, i.e. the way an individual's endogenous circadian clock rhythm runs and synchronizes (entrains) to the 24-hour day. Off-center chronotypes, i.e. individuals who are really early birds or night owls, were overrepresented among migraine patients. Patients were more often early or late chronotypes as compared to the control group of non-headache subjects. As only few small studies had investigated this association before [32, 35], this study established the link between migraine (with and without aura) and off-center chronotypes. Interestingly, these data underlined that migraine attacks have a preponderance to strike in the early morning. In addition, for the first time, it was described that migraine patients can cope less well with alterations in circadian rhythm: they are less flexible and more rigid in adjusting the setting of their biological clock. These observations underscore an important role for chronobiological mechanisms in migraine attack initiation. Whether training or entrainment of the biological clock has an improving effect on migraine attack frequency or duration still needs to be established.

In **chapter 4** an increased prevalence of restless legs syndrome (RLS) in migraine patients

vs. controls was described, consistent with previous findings [5-11]. Interestingly, RLS was also found to be more severe among the migraine patients, and interfere more with sleep quality, thereby possibly moderating an additional triggering factor for migraine attacks. Migraine severity was correlated with RLS-severity. A number of hypotheses has been presented to explain the association between RLS and migraine [6]: dopaminergic dysfunction, dysfunctional iron metabolism, genetic linkage and sleep disturbances. RLS has since long been considered to be related to dopaminergic system dysfunction [12-19], and the A11 dopaminergic nucleus of the dorsal-posterior hypothalamus is hypothesized to be involved in both RLS [19-22] and migraine pathophysiology [23-25]. Although treatment of RLS with dopamine agonists leads to rapid and dramatic improvement in symptoms with dopamine agonists [26], the effect on migraine headache relief is not clear [27-29]. Whether treating migraine affects concomitant RLS symptoms also still needs to be established.

Post-dural puncture headache and experimental trigger factors for migraine attack initiation

In **chapter 5** it was described that migraineurs are not at increased risk of developing post-dural puncture headache (PDPH), in contrary to what was generally assumed before. The incidence and severity of PDPH were studied as part of an extensive biochemical migraine research programme in which cerebrospinal fluid sampling was performed using a defined protocol in 160 migraine patients and 53 age- and sex-matched healthy non-migraine control subjects. In total, 64 of 199 subjects (32.2%) with successful lumbar puncture developed PDPH. Young age, low body mass index, severe headache immediately after LP, and sitting sampling position, but not being a migraineur, increased the risk of PDPH. PDPH duration was prolonged by history of depression, sitting sampling position, high perceived stress during the procedure, and multiple lumbar puncture efforts. PDPH duration was found to be similar in migraineurs and control subjects. Furthermore, migraine attacks were less likely to occur before or shortly after lumbar puncture. This suggest that a lumbar puncture is not a trigger for a migraine attack, and that the stress of an upcoming lumbar puncture might even have a protective effect against onset of migraine attacks.

Based on the epidemiologic observation that migraine and vasovagal syncope are comorbid conditions and on the experimental observation that nitroglycerin can trigger both syncope and migraine attacks, in the study described in **chapter 6** the migraine-specific effects of intravenous nitroglycerin infusion on cardiovascular parameters were assessed. First, after nitroglycerin infusion, patients in whom an attack was provoked showed a stronger cardiovascular response compared to the non-migraine controls. Secondly, in patients who showed a stronger cardiovascular response in heart rate, stroke volume, and cardiac output during nitroglycerin infusion, a shorter time until the provoked migraine attack initiated was observed. Migraine patients in whom no attack was provoked had weaker cardiovascular responses, similar to the responses in the control subjects. The stronger systemic cardiovascular responses in migraine suggest increased systemic sensitivity to vasodilators, possibly due to insufficient autonomic compensatory mechanisms. As these autonomic mechanisms are partly controlled by hypothalamic signalling, a role for hypothalamic disfunctioning in attack susceptibility can be postulated.

Part II: Imaging aspects

Even though clinical evidence suggests hypothalamic involvement during the early phases of a migraine attack, very few imaging studies have provided evidence for this hypothesis. Only one functional Magnetic Resonance Imaging (fMRI) study focussing on the hypothalamus in migraine without aura attacks has been conducted. Three elaborate prospective studies have reported on activation of the hypothalamus during the migraine attack. Taken together, two out of these three studies provided insight in hypothalamic activity during the pre-ictal phase [36, 37], but control groups were lacking and generalizability was hampered by very small numbers [36-38]. Furthermore, they all used indirect measures for hypothalamic metabolic changes: either the Blood Oxygen Level Dependent (BOLD) signal or perfusion changes as measured with $H^2_{15}O$-PET as a proxy for neuronal activity.

Altered hypothalamic neuronal activity as measured with fMRI

In **chapter 7** an altered hypothalamic neuronal activity in response to an oral glucose load was reported in the early phases of nitroglycerin-triggered and spontaneous migraine attacks based on a study using functional Magnetic Resonance Spectroscopy (fMRI). Normally, the activity of the hypothalamus will remain low after ingestion of glucose, probably reflecting a 'satisfied' state of the glucosensitive neurons in the lateral hypothalamus [41, 42]. In the migraine group, the hypothalamus seemed not to respond to glucose during the premonitory phase, and activity did not show the normal, glucose-induced drop. The abnormal, prolonged increase we found in the migraine group might then reflect disinhibited satisfaction, which could link to the common premonitory symptom of craving. However, as the hypothalamus controls different homeostatic mechanisms, linking this abnormal response to one specific symptom would be an oversimplification. Our study is the first study showing an abnormal response of the fMRI-signal in the hypothalamus during the earliest phases of both nitroglycerin-provoked and spontaneous migraine attacks and corroborates the hypothesised role of the hypothalamus very early in the migraine attack, and additional studies are necessary to elucidate the precise role of the hypothalamus.

Part III: Biochemical aspects

In the third part several biochemical modulators of migraine attack onset in both male and female patients are described. Migraine prevalence and the frequency, duration and severity of migraine attacks are highly dependent on age, gender and, in women, events which are associated with marked fluctuations in the female reproductive hormones oestrogen and progesterone [43-45]. Testosterone suppletion had been suggested to modulate migraine frequency [46].

Blood hormone levels

Although there was considerable evidence from epidemiological, biochemical and experimental studies that female sex hormones might modulate migraine prevalence, frequency and severity in women, the role of oestradiol and testosterone in migraine

susceptibility and attack onset in male patients had not been studied. In **chapter 8** we described our prospective study into interictal, pre-ictal and ictal levels of 17ß-oestradiol and free testosterone in men with migraine and analysed the temporal relationship of changes in these levels with the occurrence of attacks. We found that interictally, male migraine patients have increased levels of 17β-oestradiol compared to non-headache controls, and that levels of free testosterone increase during the pre-ictal phase in patients who report clinical premonitory symptoms. Furthermore, we presented evidence for a relative androgen deficiency in the migraine group. Only one previous study had investigated testosterone levels in a total of eight male migraineurs. In accordance with our results, testosterone levels outside migraine attacks in that study did not differ from baseline [47]. We hypothesise that the pre-ictal rise in testosterone could reflect a general stress-response anticipating the impending attack [48].

Several mechanisms are proposed by which changes in the levels of reproductive hormones could modulate migraine susceptibility. The late-luteal phase drop in 17ß-oestradiol specific to patients with menstrual-related attacks [45, 49] is suggested to affect the balance between long-lasting genomic effects of nuclear oestradiol receptors and short-lasting non-genomic effect via intra-membranous G-protein-coupled oestradiol receptors [50]. This imbalance could induce neuronal sensitisation and ultimately triggering of attacks [50]. Secondly, female and male sex hormones differentially affect two important basic mechanisms likely involved in migraine pathogenesis as noticed in animal experiments. Female gonadal hormones lower the threshold for inducing cortical spreading depressions (CSDs). CSD is a putative surrogate marker of migraine susceptibility and is considered the biological correlate of the migraine aura [51]. Furthermore, female sex hormones activate the nociceptive transmission within the trigeminovascular system by modulating expression of nociceptive mediators such as calcitonin gene-related peptide (CGRP) and by affecting serotonin synthesis, dural mast cell density, and intracellular downstream signalling [51, 52]. Taken together, what exactly is the role of oestradiol in men with migraine and whether fluctuations in oestradiol levels, like in women, might be associated with changes in migraine activity, deserves further intra-individual follow-up studies over multiple attack cycles.

Trigeminal cyclicity and calcitonin gene-related peptide (CGRP) [ref paper]

Calcitonin gene-related peptide (CGRP) is a potent vasodilator and a key mediator in migraine [53], which is released from the primary afferents of the trigeminal ganglion exerting its effect via the trigeminovascular system [54]. In **chapter 9** a human model to quantitatively assess salivary CGRP secretion using capsaicin as a provocative agent is described. Capsaicin induces the release of CGRP via the transient receptor potential channel V1 (TRPV1). The CGRP response after capsaicin application on the tongue was considered to reflect the "activation state" of the trigeminal nerve, since trigeminal CGRP-containing vesicles were depleted on capsaicin application. The capsaicin in our study was derived from red chili homogenate, was well tolerated and caused a dose-dependent salivary CGRP release in our pilot study. Subsequently, we validated the optimal capsaicin concentration for provocation use in a second experimental set-up. Capsaicin (-containing homogenates) had been applied in several ways before in previous studies, including subcutaneously [55], intradermally [56, 57],

topically[58, 59] and orally[60], but we were the first study to quantify the salivary CGRP release. This model could be helpful in elucidating the pathophysiologic mechanisms in diseases in which the trigeminal system is involved.

Given the relationship between migraine attack incidence and fluctuations in sex hormones, in **chapter 10** the hypothesis was tested whether varying levels of sex hormones over the course of the menstrual cycle affect trigeminal nerve-mediated vasodilatation of the small vessels in the forehead, a dermatome innervated by the trigeminal nerve. In a prospective study, we compared dermal blood flow (as a proxy for vasodilation) during the mid-luteal phase and during menstruation phase after both topical capsaicin application and electrostimulation in patients with menstrual-related migraine, in age-matched healthy controls with a menstrual cycle, and in a reference group of post-menopausal women. In migraine we found a diminished cyclicity of dermal blood flow response to topical capsaicin application. The responses after electrostimulation and the salivary CGRP levels were similar throughout the menstrual cycle in all groups. Previous studies, however, have found elevated levels of salivary CGRP during the pre-ictal and ictal phases of the migraine attack [61, 62]. Since the time points in this study were not based on clinical migraine attack phase (but rather on menstrual cycle timing) no conclusions with regard to the salivary CGRP levels could be drawn. This study corroborated the hypothesis that the premenstrual decrease in oestradiol affects the trigeminovascular system, and underlined that patients with menstrual related migraine have both an altered systemic and trigeminovascular cyclicity. This might explain their augmented susceptibility to a migraine attack around the time of menstruation.

Overall resume and future perspectives

The premonitory phase and early phase of both spontaneous and nitroglycerin-triggered migraine attacks were explored in this thesis, in association with clinical modulators and trigger factors. Clinical research strategies, experimental designs, neuroimaging techniques and biochemical methods have revealed clinical risk factors, biochemical modulators and pharmacological triggers. Furthermore, newly discovered hypothalamus-specific alterations in metabolism and perfusion in the early phases of the migraine attack were described. Taken together, these results suggest that each migraine attack starts well before the initiation of the headache phase. The hypothalamus is postulated to have a pivotal role in the early phases of the migraine attack, and possibly affects attack susceptibility interictally as well.

Overall, the studies described in this thesis have improved our understanding that the migraine brain (and possibly the hypothalamus) differs from the non-migraine brain already at baseline. The studies described in this thesis also underline the importance of studying the entirety of the migraine attack: from interictal, via premonitory, to the headache phase. Longitudinal studies that collect data over these consecutive phases are of utmost necessity to further elucidate the complex biochemical alterations in the brains and bodies of migraine patients.

In depth profiling or defining of migraine patients, so-called phenotyping, either with very detailed questionnaires or face-to-face, should form the core of any future study in migraine, also when using large samples. Assessing the relationship between migraine and comorbidities or individual traits gives insight in underlying, shared mechanisms and can help identify important pathways or structures involved in migraine pathophysiology. Detailed neuro-imaging evaluation, using well-defined subjects and valid imaging techniques, can be used for studying brain structures, even those that are localized deep within the brain, such as the hypothalamus. S*tate of the art* imaging techniques are necessary since the presumed biochemical differences or changes are very small. The biochemical assessment of bodily fluids (cerebrospinal fluid, saliva, blood), provides an opportunity to study hormones, peptides or other molecules in a clinical context and with the possibility of repeated measurements over the course of a migraine attack. Therefore, performing such prospective studies in the future, although logistically very challenging, remains very important.

In conclusion, the studies in this thesis have improved our understanding of pathophysiological mechanisms in the early phase of migraine attacks and the role of the hypothalamus herein. The studies have shown that epidemiological, clinical, biochemical and imaging strategies are valid tools to attack onset mechanisms, and that longitudinal, repeated measurement study designs are well worth the logistical challenges. It remains pivotal to translate biochemical and imaging findings into novel treatment options for migraine patients.

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