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High brain serotonin levels in migraine between attacks: A 5-HT_4 receptor binding PET study

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A B S T R A C T

Migraine has been hypothesized to be a syndrome of chronic low serotonin (5-HT) levels, but investigations of brain 5-HT levels have given equivocal results. Here, we used positron emission tomography (PET) imaging of the 5-HT_4 receptor as a proxy for brain 5-HT levels. Given that the 5-HT_4 receptor is inversely related to brain 5-HT levels, we hypothesized that between attacks migraine patients would have higher 5-HT_4 receptor binding compared to controls. Eighteen migraine patients without aura (migraine free > 48 h), and 16 age- and sex-matched controls underwent PET scans after injection of [^{11}C]SB207145, a specific 5-HT_4 receptor radioligand. An investigator blinded to group calculated a neocortical mean [^{11}C]SB207145 binding potential (BP_{ND}). Three migraine patients reported a migraine attack within 48 h after the scan and were excluded from the primary analysis. Comparing 15 migraine patients and 16 controls, we found that migraine patients have significantly lower neocortical 5-HT_4 receptor binding than controls (0.60 ± 0.09 vs. 0.67 ± 0.05, p = .024), corrected for 5-HTTLPR genotype, sex and age. We found no association between 5-HT_4 receptor binding and attack frequency, years with migraine or time since last migraine attack. Our finding of lower 5-HT_4 receptor binding in migraine patients is suggestive of higher brain 5-HT levels. This is in contrast with the current belief that migraine is associated with low brain 5-HT levels. High brain 5-HT levels may represent a trait of the migraine brain or it could be a consequence of migraine attacks.

1. Introduction

Migraine is a highly debilitating and socioeconomically costly neurological disorder, affecting 16% of the population worldwide (Olesen et al., 2012; Steiner et al., 2013). Despite intensive research during the past several decades, the neurobiological basis and pathophysiology of migraine remains largely unknown. Serotonin (5-hydroxytryptamine, 5-HT) has been directly implicated in the pathophysiology of migraine (Hamel, 2007) and studies on plasma and urinary levels of 5-HT and its main metabolite, 5-hydoxyindoleacetic acid (5-HIAA) suggest that between their migraine attacks, patients have decreased levels of plasma 5-HT (Ferrari et al., 1989; Sicuteri et al., 1961). Accordingly, although plasma levels of 5-HT do not necessarily reflect brain 5-HT levels, migraine has been considered a syndrome of chronically low brain 5-HT levels. Several studies have attempted to assess brain 5-HT levels in migraine patients, but results have been equivocal, showing both higher and lower levels compared to controls (Deen et al., 2017a). We here use a novel neuroimaging method to investigate if migraine is a syndrome associated with low 5-HT brain levels.

The 5-HT_4 receptor, one of 14 5-HT receptors, is inversely related to central serotonergic tonus and can thus be used as an indirect biomarker of brain 5-HT levels. In rats, brain 5-HT_4 receptor binding decreased after 14 days of selective 5-HT reuptake inhibitor (SSRI)

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administration (Licht et al., 2009). In humans, carriers of the short allele of the 5-HT transporter (5-HTT) gene, which is associated with relatively increased synaptic 5-HT levels, had lower neocortical 5-HT_4 receptor binding compared to carriers of the long allele (Fisher et al., 2012). Furthermore, the final support for the 5-HT_4 receptor being inversely related to brain 5-HT levels, came from a study showing that three weeks of SSRI intervention led to a significant decrease in brain 5-HT_4 receptor binding in humans (Haahr et al., 2014).

Here we investigated differences in brain 5-HT levels between migraine patients without aura and controls using PET imaging of the 5-HT_4 receptor as an in vivo biomarker of brain 5-HT levels. According to existing beliefs, we hypothesized that migraine patients had higher 5-HT_4 receptor binding compared to controls.

2. Materials and methods

2.1. Subjects

Participants were recruited through a Danish website for recruitment of volunteers to health research and from a local database. All patients fulfilled the following inclusion criteria: 1) 18–65 years old, 2) a verified diagnosis of migraine without aura according to the International Headache Society Criteria (HCC IHS, 2013). 3) at least one migraine attack every other month but less than five migraine days per month, 4) self-reported previous effect of treatment of migraine attacks with sumatriptan (a 5-$HT_{1B/1D}$ receptor agonist drug). The last criterion was applied, since the subjects were also included in a study investigating the 5-HT_{1B} receptor (Deen et al., 2017b). A standardized interview of all patients was conducted at screening including the following items: duration of disease (years), duration of attack when untreated (hours), migraine days pr. month, frequency of attack (number per month), maximum pain intensity of untreated headache as measured with the Numerical Rating Scale (NRS) (number 0–10), intake of acute pain medication including triptans (days per month), and date of their last migraine attack. Inclusion criteria for age- and sex-matched controls included: 1) no history of migraine including probable migraine and no first-degree relatives with migraine. For all participants, the following exclusion criteria were applied: 1) a history of any other primary headache (except tension-type headache < 5 days per month), 2) psychiatric, cerebro- or cardiovascular disease, 3) contraindications for magnetic resonance imaging (MRI), 4) pregnancy or nursing, 5) daily intake of medication including migraine prophylaxis.

All subjects reported to be headache free on the day of their PET scan, and no medication intake was allowed for the last 24 h prior to the scan. All migraine patients were migraine free for at least 48 h prior to the PET scan. In addition, to ensure that all included subjects were truly between two migraine attacks, headache diaries were obtained from all patients for 48 h after the scan. All included participants had a normal physical and neurological examination and unremarkable brain MRI. All participants filled out the major depression inventory (MDI) on the day of the PET scan.

The study was approved by the Ethics Committee of The Region of Copenhagen (H-6-2014-057). In accordance with the Declaration of Helsinki of 1964, with later revisions, all participants gave written consent after detailed oral and written information about the study.

2.2. PET and MR imaging

Synthesis of the radioligand, [^{11}C]SB207145, was performed using an automated radiosynthesis system as previously described (Marner et al., 2009). An intravenous bolus injection of the radioligand was given over 20 s, followed by 120-minute dynamic data acquisition with a high-resolution research tomography (HRRT) PET scanner (CTI/Siemens, Knoxville, TN, USA). To minimize head movement, all subjects had their head stabilized in a specialized head holder. The scans were reconstructed into 38 frames (6 × 5, 10 × 15, 4 × 30, 5 × 120, 5 × 300, and 8 × 600 s) using a 3D-OSEM-PSF algorithm (16 subsets, 10 iterations) with TXTV based attenuation correction (image matrix, 256 × 256 × 207; voxel size, 1.22 × 1.22 × 1.22 mm), as previously described (Hong et al., 2007; Keller et al., 2013; Sureau et al., 2008). T1 and T2 weighted MRI scans used for co-registration were acquired for each subject using a Siemens Prisma 3T scanner (Siemens, Erlangen, Germany) with a 64-channel head coil.

2.3. Quantification of 5-HT_4 receptor binding

Single-subject PET images were corrected for intra-scan movement by aligning the frames 10–38 to a reference frame (frame 26) using a scaled least-squares cost function in AIR 5.2.5. Co-registration and alignment of PET images to the corresponding T1-weighted MRI image was done using SPM8. Regions of interest (ROIs) were automatically delineated on each subject's MRI using PVElab software (www.nru.dk) as previously described (Svarer et al., 2005). Accurate co-registration and ROI placement were confirmed by visual inspection for each subject, across all planes. Time activity curves (TAC) and grey matter volumes for each ROI were then extracted.

The Simplified Reference Tissue Model (SRTM) was applied to calculate the non-displaceable binding potential (BP_{ND}) of [^{11}C]SB207145. This model has previously been validated for quantification of [^{11}C]SB207145 in the human brain (Marner et al., 2009). Cerebellum (excluding vermis) was used as a reference region since it has a negligible density of 5-HT_4 receptors (Ganz et al., 2017; Marner et al., 2009). Kinetic modeling was performed in MATLAB R2013a (8.1.0.604) 64 bit (Mathworks Inc., MA) using an in-house script and the person performing the kinetic modeling was blinded to group status (migraine patient or control). Parametric 5-HT_4 receptor binding images for voxel based analysis were generated using PETSurfer (http://surfer.nmr.mgh.harvard.edu, version 6.0), as previously described (Greve et al., 2014). In summary, a combined volumetric and surface registration algorithm was used to normalize each single-subject structural T1 to Montreal Neurological Institute (MNI) space (Postelnicu et al., 2009). After application to the co-registered PET-images, these were then volume-smoothed with a 6-mm full-width half-maximum 3D Gaussian kernel. The Multilinear Reference Tissue Model 2 (MRTM2), using cerebellum as reference region and high-binding regions (putamen, pallidum and caudate) for estimation of k_2', was applied to estimate voxel-level BP_{ND}s.

2.4. Genotyping

Participants were genotyped for the tri-allelic 5-HT transporter-linked polymorphic region (5-HTTLPR) polymorphism. Briefly, genotyping was performed by PCR amplification from forward primer 5′-TAATGTCCCTACTGCAGCCC-3′ and reverse primer 5′-GGGACTGAG CTGGACAACC-3′. The fragments were then digested by the restriction enzyme MspI and separated by gel electrophoresis. Participants were categorized into two groups: 1. Carriers of the short allele (S-carriers) or the long L_G allele (L_G-carriers). 2. Homozygotes of the long L_A allele (L_A-homozygotes). This dichotomization was based on a previous study showing that carriers of the low-expressing alleles, S and L_G have lower neocortical 5-HT_4 receptor binding compared to homozygotes of the high-expressing allele, L_A (Fisher et al., 2012).

2.5. Experimental design and statistical analysis

This study was an observational, cross-sectional study comparing interictal migraine without aura patients (> 48 hour migraine free before and after the scan) with sex- and age matched controls. Sample size was based on a previous study showing that n = 15 is sufficient to detect a 15% difference between groups with a power of 0.80 in very large brain regions (> 50 cm^3), such as e.g. neocortex (Marner et al., 2010). Differences between groups in demographics, genotypes and