Effects of glyceryltrinitrate and calcitonin gene-related peptide on BOLD signal and arterial diameter:

- Methodological studies by fMRI and MRA

Mohammad Sohail Asghar

This review has been accepted as a thesis together with three previously published papers by University of Copenhagen the1. November 2011 and defended on 23.february 2012.

Tutors: MessoudAshina & Henrik B.W. Larsson

Official opponents: Arne May, Flemming bach & Hanne Christensen

Correspondence: Department, Danish Headache Center, Dep. Of Neurology, Glostrup Hospital, Nordre Ringvej 57, 2600 Glostrup, Denmark

E-mail: Sohail@dadlnet.dk

Dan Med J 2012;59(7):B4489

THE THREE ORIGINAL PAPERS ARE:

- Pharmacological modulation of the BOLD response – a study of acetazolamide and glyceryl trinitrate in humans. J Magn Reson Imaging. 2011 Oct;34(4):921-7.
- Dilatation by CGRP of middle meningeal artery and reversal by sumatriptan in normal volunteers. Neurology. 2010 Oct 26;75(17):1520-6.
- Effect of CGRP and sumatriptan on the BOLD response in visual cortex, J Headache Pain. 2012 Mar;13(2):159-66

INTRODUCTION

Advances in brain imaging have substantially increased our understanding of structural and functional networks in the human brain [1]. In the early 1970's the advent of computerized axial tomography (CAT scans) revolutionized the field of neurology and neuroscience. Almost concurrently magnetic resonance imaging (MRI) was developed but first introduced into clinical work in the 1980's [1]. At present, brain imaging is an integral part of neurological diagnostics.

Brain imaging is divided into structural and functional modalities. Structuralimaging techniques such CAT scan provide anatomical information only. Functional imaging techniques such as single photon emission computed tomography(SPECT) or positron emission tomography (PET) allows recordings of brain function and/or metabolism but poor anatomical images. The major technological advantage of MRI is that this technique provides both detailed structural images and functional activation maps of the brain [2]. The multi-modal capabilities of MRI have been successfully used in exploring the mechanisms underlying neurodegenerative diseases such as schizophrenia and dementia [3].

Over the past decade functional pharmacological MRI has shown useful results in drug development and discovery [4]. Pharmacological MRI (phMRI) investigates the interaction between brain physiology, neuronal activity and drugs. Neuronal activity is recorded indirectly by measuring the associated changes in flow- and metabolism [5, 6]. Modulation of cerebral hemodynamics can therefore potentially alter the recorded neuronal signal [7], and one should be cautious in interpreting the functional MRI (fMRI) data [8, 9]. It is particularly important when fMRI recordings are performed during pharmacological perturbation, because this can modulate the neuronal signal by altering the cerebral hemodynamics. Therefore the *de novo* effect of the perturbation alone should methodological studies by fMRI and MRA be well characterized before the results can be interpreted as possible changes in brain activity to avoid false positive or false negative results.

The advent of magnetic resonance imaging angiography (MRA) has opened a new window to the understanding of cerebral hemodynamics [10, 11]. Anatomical studies have confirmed that brain arteries are complex structures involved in auto-regulation, maintaining blood supply to the brain and signal transmission [12]. Release of endogenous substances with vasoactive properties can indirectly affect these processes by inducing diameter changes in brain arteries. Administration of exogenous vasoactive substances may cause similar changes. Therefore detailed and precise information of cross-sectional changes in the cerebral arteries by high resolution MRA after administration of pharmacological substances may provide important data on the vascular effect of pharmacological substances. These data can be used for future drug discoveries including identification of drugs in pharmaceutical pre- and post marketing studies.

The overall purpose of this thesis is to explore how vasoactive pharmacological substances may influence brain hemodynamics and brain activity as recorded by MRI.

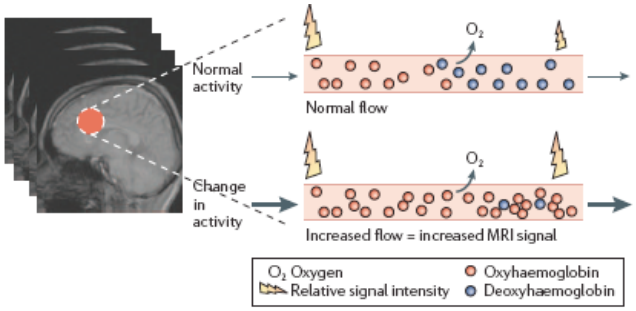
MAGNETIC RESONANSE IMAGING

Magnetic resonance imaging (MRI) is a non-invasive imaging method without ionizing radiation. Instead it uses a strong electro-magnetic field to align the nuclear magnetization of the hydrogen atoms of the water molecules in the body. Radio frequency (RF) fields are then used to systematically alter the alignment

of the magnetization. This in turn causes the hydrogen atoms to create a rotating magnetic field that can be detected by the scanner. This MR signal can be used to construct an image of the body [1].

Pharmacological MRI (phMRI) characterizes the effects of pharmacological substances by use of MRI and functional MRI (fMRI) [13]. fMRI has developed significantly during the last decade. Currently, the majority of brain mapping studies relies on fMRI rather than on radiotracer techniques. The most commonly used fMRI modality is the blood oxygenation dependent (BOLD)signal. The BOLD signal is sensitive to changes in cerebral blood flow (CBF) and deoxyhemoglobin that is associated with changes in neuronal activity. During brain activation the supply of oxygen is known to increase more than is consumed by the activated neurons. This results in a concomitant decrease in deoxyhemoglobin levels [14]. Because deoxyhemoglobin is paramagnetic, this gives a more homogenous magnetic field and this is detectable by MRI. The BOLD signal is therefore normally described as an indirect measurement of neuronal activity [6,15, 16](Fig. 1 & Fig. 2).

Figure 1:

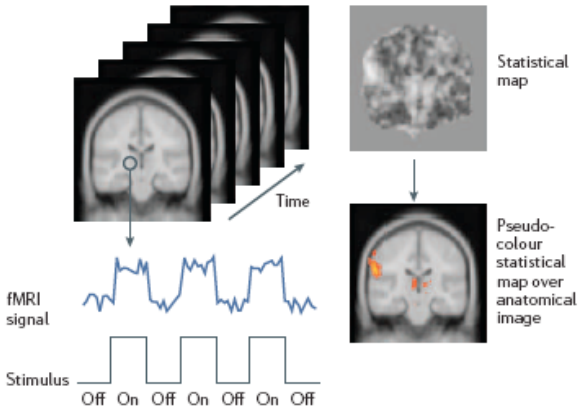


Neuronal activity triggers an increase in blood supply to the surrounding capillary beds that overcompensates for neuronal oxygen extraction, causing a relative change in their oxy/deoxyhemoglobin status; oxyhemoglobin concentration increases while deoxyhenoglobin decreases. Becauseoxy hemoglobin is less paramagnetic then deoxyhemoglobin the MR signal intensities in the area around these capillaryss change; increased concentration of oxyhemoglobin result in higher MR intensities. A net increase in MR signal intensity is therefore detected in areas that become active and depends on the magnitude of the blood-flow changes evoked. For this reason, it has been defines as blood oxygenated level dependent (BOLD) fMRI. The increased blood flow (which correlated with increased neural activity) produces fMRI signal changes in the order of 0.5-5% (from: Borsook et al. 2006)

The BOLD signal is sensitive to the total amount of deoxyhemoglobin pr. unit of tissue, which in turn is determined from a balance between oxygen supply and use. Furthermore, the BOLD signal also depends on factors such as blood flow, blood volume, hematocrit, cerebral oxidative metabolic rate ($CMRO_2$), pH, PaO_2 and the regional anatomy of the microvasculature [8]. The BOLD-signal is thereby not specific to neural activation but can possibly be influenced by factors that can alter the physiological state of the brain. It remains uncertain how isolated changes in brain hemodynamics such as isolated blood flow or blood volume may influence the BOLD signal.

MR-angiography (MRA) is a direct non-invasive method, which permits detailed images of human vessels including both intra- and extra cerebral arteries. The brain arteries are richly supplied with peri-vascular nerve fibers and are complex structures that are involved in regulation of cerebral blood flow and volume. Release of vasoactive neurotransmitters from the peri-vascular-sensory afferent nerve endings plays an important role in the regulation of the cerebral hemodynamics[17-19]. Administration of exogenous substances can be used to mimic endogenous intravascular release of neurotransmitters. The possible vascular action and site-of-action can then be identified by MRA. Finally, MRA can also be used to examine the cerebral vasculature during a pharmacological intervention against diseases.

Figure 2:



In an fMRI experiment, subjects are inserted into the MRI scanner and a series of stimuli is applied. Typically the stimuli are presented as "on" and "off" periods. During the stimulus presentation several images of the brain are acquired. One approach to detecting activation consists of correlating the temporal profile of the stimulus with the temporal evolution of each "point" (voxel) in the image. Voxels that are highly correlated with the stimulus receive a high statistical score. An image is created that reflects the statistical score for each voxel, a statistical map. The statistical map however, lacks any anatomical information that could define the structures with significant activation. Statistical maps are colour coded and overlaid on anatomical images (usually statistical maps are displayed in such a way that only voxels with a significance larger that a threshold are included and the rest are not considered/displayed). The resulting combined image allows the identification of voxels that are significantly activated and the central nervous system substrates they belong to. fMRI resolution of standard clinical 3-telsa scanners is typically about 10 mm^3 (and could be as low as 1 mm^3) (from: Borsook et al. 2006)

VASOACTIVE DRUGS AND MRI

Vasoactive drugs may be used as pharmacological tools to study the in vivo effect of hemodynamic changes in the human brain by fMRI. Drugs known to induce changes in cerebral blood flow (CBF), cerebral blood volume (CBV), vasodilatation or vasoconstriction may modulate the BOLD response. In the present thesis we used four different drugs to induce hemodynamic changes. In the following a concise review on their known effects on cerebral hemodynamics is presented:

Acetazolamide: A potent carbonic anhydrase (CA) inhibitor (CAI), is often used in clinical settings to assess cerebrovascular reserve capacity. It exerts no detectable toxic effect at physiologically useful doses (< 50 mg/kg). It is generally believed that acetazolamide increase the CBF through a transient arterial acidosis by inhibiting CA [20, 21]. It is not believed to influence blood pressure nor does it decrease the cerebral vascular tone [21, 22]. The effect of acetazolamide on the cerebral vessels is still a subject of debate.

Glyceryl trinitrate: The Nitric Oxide (NO) donor GTN is known to cause dilatation of the cerebral vessels including the middle cerebral artery (MCA) [23] and the middle meningeal artery [24]. The GTN experimental headache model is a widely known vascular headache model to induce head pain including migraine [25]. With the dilatation of the cerebral vessels GTN is believed to cause an increase of CBV without affecting the CBF. GTN also causes a decrease in systemic blood pressure [25].The effect of GTN on the BOLD response has not previously been studied.

Calcitonin gene-related peptide (CGRP): CGRP is a widely distributed neuropeptide in perivascular nerve fibers [26] and is recognized as one of the most powerful vasodilators [27-29]. Its receptor components are found in the smooth muscle cells of cranial arteries [30] and exogenous CGRP is believed to interact with the smooth muscle cells. There is controversy regarding CGRP's effect on CBF [31, 32]. Based on Doppler ultrasound studies it is believed that CGRP dilates intra-cerebral arteries. The effect of CGRP on dural arteries and brain activity has previously not been studied. Infusion of human alpha CGRP (h-alpha CGRP) induces immediate headache in healthy volunteers [33] and migraineurs [34]. Mechanisms responsible for the initiation of CGRP induced headache or migraine are not fully clarified.

Sumatriptan: A 5-HT1b/1D receptor agonist was originally developed as a selective cranial vasoconstrictor for treatment of migraine [35]. However, the mode of action of sumatriptan is far more complex than initially perceived. It has been suggested that sumatriptan mimic its effects through inhibiting the release of CGRP. Previous studies using indirect ultrasound based measurements have shown that sumatriptan contracts the middle cerebral artery and the superficial temporal artery (STA) [35, 36]. The effect of sumatriptan on the extracerebral middle meningeal artery (MMA) and on the BOLD signal has not previously been studied in humans.

SPECIFIC AIMS

- To examine the effect of CBF and CBV changes induced by acetazolamide and GTN on the BOLD signal.
- To examine the vascular-site-of-action of CGRP on the intracranial arteries and to examine the effect of sumatriptan after and outside of pre-treatment with CGRP.
- To examine the effect of CGRP on the BOLD response and to examine the effect of sumatriptan on the BOLD response outside and during treatment with CGRP.

METHODS

VOLUNTEERS (STUDY I, II & III)

The study protocols were approved by the local Ethical Committee of Copenhagen. We recruited healthy volunteers (age 18 – 55years) through posters on the hospital and faculty grounds and via advertisement on the internet. The volunteers were excluded if they: had a history of migraine or any other type of headache(except episodic tension-type headache < 5 days/month); took any daily medication apart from oral contraceptives; had serious somatic (including any history or sign of asthma) or psychiatric disease. 6 subjects were recruited for 2study I, while 18 subjects were requited for study II and III.

EXPERIMENTAL DESIGN STUDY I-III

All studies were conducted using a balanced randomized double-blind crossover design. All participants reported to the laboratory headache free. After baseline measurements, infusion started using a time and volume controlled infusion pump(Braun Perfusor, Melsungen, Germany). Magnetic resonance imaging (MRI) were preformed on a 3.0 Tesla Philips Achiva scanner (Philips Medical Systems, Best, The Netherlands) using an eight-element phased-array receive head coil. Anatomical images were acquired using a T1-weighted 3D turbo field echo sequence (170sagital slices 1 mm thick; in-plane resolution 1x1 mm: repetition time 9.9 s; echo time 4.6 ms; flip angle 80^{o}).

In study I six volunteers (3F and 3M, mean age 24 years, Range 21-31 years) were randomly allocated to receive either GTN (0.5 mg/kg/min), placebo (normal saline)or acetazolamide (1 g bolus injection) on total three study days. The study days were separated by least 7 days to avoid carry-over or period effects. BOLD-measurements were obtained from the visual cortex.

In study II & III 18 volunteers (11F and 7M, mean age 25 years (range 22-28years)) were randomly allocated to receive 1.5 mg/min CGRP (Calbiochem, Merck4BiosceincesR, Darmstadt, Germany) or placebo over 20 min on two days, separated by least 7 days. On both study days the participants received subcutaneous injection of 6 mg sumatriptan 45 min after start of infusion. Arterial diameters were recorded by MRA at baseline (T_{-10min}) and, T_{30}, T_{60} after start of infusion. The BOLD signals were recorded by fMRI at baseline and T_5, T_{10}, T_{15} T_{25}, T_{40}, T_{50},T_{60} and T_{75} after start of infusion.

FMRI

In Study I and III the blood oxygenation level dependent (BOLD) signal was measured as an indirect measurement of neuronal activity in the brain. BOLD functional imaging utilized a gradient echo EPI sequence (32 slices 4.0 mm thick; slice gap 0.1 mm; field of view 230 x 230 mm; in-plane acquired resolution 2.9 x 2.9 mm; repetition time 3.0 s; echo time 35 ms; flip angle 90^{o}; SENSE factor 2). Slices were oriented parallel with the inferior border of corpus callosum covering the whole brain. The first four volumes of each run were discarded to avoid saturation effects. We obtained 100 volumes during each stimulus.

As region of interest (ROI) the visual cortex was chosen. To record the BOLD response we applied visual stimulation with a checkerboard. This is a well-established modality that produces a rather large BOLD response in the visual cortex [37, 38]. Visual stimulation was presented with the Eloquence system (Invivo, Orlando, Florida), using a pair of NNL goggles (Nordic Neuro Lab, Bergen, Norway). A fiber optic cable connected the system to a control computer outside the scanner room. The paradigm consisted of rest blocks, where a uniform grey image was shown, alternating with active blocks displaying a black and white checkerboard reversing at 8 Hz. The block length was 1min and two activation periods where included during a scan session which had a duration of 5 min. Subjects were asked to fixate on a central fixation cross during the entire scan. The onset of visual stimuli was triggered by the scan acquisition. In study III eye tracking (Nordic Neuro Lab (NNL) was applied to monitor the subjects.

MRI ANGIOGRAPHY

In study II the MRA was used to measure diameter of the MMA and the MCA. For vessel imaging, a 3D inflow gradient echo sequence was used. First, a scout MRA was preformed using field-of-view (FOV) 180x180x180; acquired matrix size (M x P) of 180x180, acquired voxel resolution 1.0x1.0x2.4mm^3, reconstructed resolution0.34x0.34x1.20 mm^3, repetition time (TR) 23ms; echo time (TE) 5.4 ms; flip angle 20^{o}; SENSE factor 2;5 chunks, total scan duration of 198 sec. The scout MRA was used to plan the subsequent MCA scans. The MRA of MCA utilized: FOV 200x200x74 mm^3; acquired matrix size (M x P) of 800x406, acquired voxel resolution 0.25x0.49x1.00mm^3; reconstructed resolution 0.20x0.20x0.5mm^3; TR: 25ms; TE: 3.5ms; flip angle 20^{o};

SENSE factor 2;4 chunks, total scan time of 9 min02 sec. The first acquired angiography (base-line) had a large vertical FOV to ensure that the MCA was imaged, and was used to place the subsequent angiography of MMA. The remaining MCA scans had a FOV of 200x200x20mm^3. MMA scans had FOV 200x200x16.1mm^3, acquired voxel resolution of 0.25x0.35x0.70mm^3;reconstructed resolution 0.2x0.2x0.35mm^3,TR: 25ms; TE: 3.5ms; flip angle 20^o;SENSE factor 2;4 chunks, total scan duration of 328 sec.

VITAL SIGNS

Heart rate (HR), blood oxygen saturation, ECG and blood pressure (BP) were monitored continuously during the study using Veris monitor (Medrad, USA).End-tidal CO_2 was monitored using a capnograph (Datex, Helsinki Finland).

ADVERSE EVENTS

Adverse events (AE's) such as headache, nausea and other sensations relating to drug side-effects were recorded continuously during the studies. Headache intensity was recorded repeatedly on a verbal rating scale (VRS) from 0 to 10 [0, no headache; 1, a very mild headache (including a feeling of pressing or throbbing pre-pain), 10, worst imaginable headache) [39]. Headache characteristics and associated symptoms were also recorded to determine the quality and type of the headache.

BOLD DATA ANALYSIS AND STATISTICS

STUDY I

In study I the analysis was performed using BrainVoyager 1.9 (Brain Innovation B.V., Maastricht, The Netherlands). Image preprocessing started with a 3D rigid body motion correction and correction for scan-time. After 3D spatial smoothing with a 6 mm FWHM Gaussian kernel, the functional images were co-registered to the 3D anatomies, transformed into Talairach space and resampled to 3 mm^{2}voxels.

The primary end-point was difference in relative BOLD response between active drug and placebo. The secondary end-point was difference in incidence of AE's between experimental days.

For the BOLD fMRI statistical-analysis a general linear model was used. The visual block stimulation paradigm convolved with a 2 gamma hemodynamic response function served as a model time course. In a second-level analysis of the beta-values, we computed a two-way ANOVA with regard to scan-time and drug for GTN versus placebo and acetazolamide versus placebo separately. The F-test statistic was calculated both voxel-wise and for a ROI. A ROI for the visual stimulation was defined by a conjunction of activation areas in all individuals from the first scan (before drug administration), which were in turn determined by requiring a false discovery rate (FDR) P<0.02 to correct for multiple comparisons. All values are presented as mean±SD. In addition, hemodynamic peak responses are presented as mean percentage from baseline [95% confidence interval (CI)].

All baseline variables were tested for period and carry-over effects using independent t-test. McNemars test was used to test difference in incidence of adverse events (AE's) between experimental days.

STUDY II

The angiography data in Dicom format was analyzed by the LKEB-MRA Vessel wall analysis software (Leiden, The Netherlands) [40]. The software provides automated contour detection and quantification of the luminal boundaries in vessel segments. The MMA was identified by marking the branch from the main trunk of the maxillary artery and the MCA by marking the branch from the main trunk of the internal carotid artery. In each scan the exact same starting and ending point was identified on each side. The software automatically calculated a path line and measured the circumference of the vessel every 0.2mm perpendicular to the center-line, from which the average circumference for each vessel was finally obtained. The primary endpoints were differences in circumference of the MMA and MCA after infusion between CGRP and placebo days. In addition we preformed explorative analysis to determine the magnitude of CGRP induced dilatation versus possible placebo induced dilatation. The secondary end-points were differences in MMA and MCA circumference after sumatriptan and between study days. To explore possible selective effect of sumatriptan we compared the relative changes between MMA and MCA on both days. Furthermore we tested for differences in headache between the two study days.

For analysis of the difference in artery circumference, we calculated the difference between time points (T_{30}- T_{-10} to test post infusion dilatation; T_{60}-T_{30} to test post sumatriptan contraction) to obtain a summary measure. To determine possible differences in sumatriptan-induced contraction between experimental days the ratio between sumatriptan induced contraction and CGRP or placebo induced dilatation was calculated and compared. To explore possible selective effect of sumatriptan we compared of the relative changes between MMA and MCA on both days. In addition, we tested the difference in the AUC for headache score in the period 0-75 min between the two experimental Conditions. The AUC for headache score after sumatriptan (45-75 min) between CGRP and Placebo days was compared after correction of headache prior to sumatriptan administration (the AUC 0 – 45 min) to test the isolated effects of sumatriptan on the headache score between two days. The area under the response curve (AUC) for headache score was calculated according to the trapezium rule [41]. All values are presented as mean ± SD, except ratio calculation (mean ± SEM), headache scores (median and quartiles) and the relative changes in vascular variables (mean and 95% confidence intervals, CI). We tested for period and carry-over effects by an independent samples t-test. To test for difference between two experimental conditions we used a paired, two-way Student's t-test, except difference in headache scores, which was tested using Wilcoxon Signed Rank Test. To test difference in adverse events between experimental days we used McNemars non-parametric test.

Five percent (*P < 0.05*) was accepted as the level of significance. All analysis was done using SPSS for Windows 16.0 (Chicago, Illinois, USA).

STUDY III

Functional images were analyzed using FMRIB Software Library (FSL) version 5.98Oxford, UK (www.fmrib.ox.ac.uk/fsl). FMRI Expert Analysis Tool (FEAT, version5.98) was used for preprocessing (first level analysis). Pre-processing steps included motion-correction, brain extraction, and spatial (4 mm smoothing) and temporal filtering (high pass 200 sec). A full quality as-

surance (QA) was done prior to the statistical analysis. Scans with severe distortions and/or excessive motion (> 3 mm) were excluded from further analysis. Those that passed QA were included in the following statistical analysis. Statistical results were co-registered first to the subjects own T1-weighted 3D anatomical images and subsequently to a standard Montreal Neurological Institute (MNI-152) atlas. The 3D anatomical images were transformed to match the dimensions of the functional scans using FSLSWAP and brain extraction was preformed using the FSL Brain Extraction Tool(BET) (fractional intensity threshold: 0.6, threshold gradient of -0.1, and robust brain center estimation). For registration to the 3D anatomical images: linear registration, full search and 9 degrees of freedom (DOF) was used, whereas 12 DOF was used for the subsequent registration to the standard MNI-152 atlas. The visual block stimulation paradigm convolved with a 2 gamma hemodynamic response function served as a model time course. Z (Gaussianised T/F) statistical images were thresholded using clusters determined by Z>2.3 and a (corrected) cluster significance threshold of *(P=0.05)*. The BOLD response to visual stimulation was extracted both as region-of-interest (ROI) expressed as COPE1-values (contrast of parameter estimates) and voxel-wise. For the voxel-wise analysis the FEAT tool was used (FEAT FSL version 4.1.6, Oxford for Mac). For the ROI analysis the visual cortex (V1, V2 and V3) was identified based on the Juelich Histological Atlas and normalized to the MNI structural atlas (Feat query FSL, version 4.1.6, Oxford for Mac). The extracted values were then transferred to SPSS 18.0 for Mac (IBMSPSS, New York, USA) and baseline corrected before further statistical analysis.

All values are presented as mean ± SD and hemodynamic peak responses as mean percentage from baseline [95% confidence interval (CI)] except vascular data (blood pressure, heart rate, end-tidal P_{CO2}, oxygen saturation), which are presented as mean ± SEM.

The primary end-points were: changes over time in relative BOLD response after infusion of CGRP or placebo; difference in BOLD response between two experimental days; difference in BOLD response before and after sumatriptan administration and between experimental days.

We analyzed for changes over time for each experimental day separately with analysis of variance (ANOVA) with the fixed factors subjects and time. To reduce mass significance the following time points were selected for analysis (T_{-5}, T_5, T_{15},T_{25} and T_{40}). Baseline was defined as T_{-5}. A second level analysis was preformed to test CGRP versus placebo using a two-way ANOVA with the fixed factors time nd drug. The sumatriptan phase of the CGRP day and the placebo day was analyzed in a similar way. Analysis for changes over time was preformed for each day separately using ANOVA with subjects and time as fixed factors. The measured time points T_{50}, T_{60} and $T_{75}$was compared against the functional scan at immediately preceding sumatriptan administration (T_{40}). A second level analysis was preformed to compare the two study days.

We tested for period and carry-over effects for baseline hemodynamic variables using independent t-test. Five percent *(P<0.05)* was accepted as the level of significance.

ADVERSE EVENTS

Adverse events were recorded and the differences between experimental days were tested by McNemars non-parametric test. In addition, we calculated the area under the curve (AUC) for headache intensity according to the trapezium rule [41]to test the difference in headache intensity between experimental days using Wilcoxon Signed Rank Test.

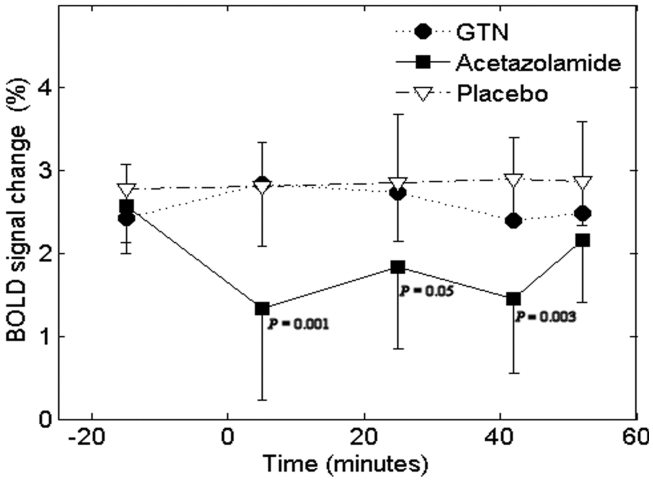
RESULTS

STUDY I

Pharmacological modulation of the BOLD response – a study of acetazolamide and glyceryl trinitrate in humans

Two-Factor ANOVA with repeated measures revealed a significant interaction between acetazolamide and placebo *(P = 0.011)*. Further analysis showed significant changes over time after acetazolamide *(P = 0.0066)* but not after placebo *(P =0.74)*. Post hoc pair-wise t-tests showed significant decrease in BOLD response at 5min *(P = 0.001)*, 25 min *(P = 0.05)* and 45 min *(P = 0.003)* after acetazolamide administration compared to baseline. The BOLD response did not return to baseline during the observation period but the last recording at 55 min did not show statistical difference compared to baseline *(P = 0.092)*. The peak decrease occurred at 5 min and was 51.9% (CI, 22.03 – 81.76) (Fig.3). The voxel-vise ANOVA did not show significantly activated voxels. Two-Factor ANOVA with repeated measures revealed no interaction between GTN and placebo *(P = 0.45)*. The voxel-vise ANOVA did not show significant voxel activation (Fig.3).

Figure3:



BOLD percent changes as function of measurement time relative to the time of start of injection. Acetazolamide significantly depressed BOLD responses at 5 min (P = 0.001), 25 min (P = 0.05) and 45 min (P = 0.003) compared to baseline. We found no statistical significant change in BOLD responses after glyceryl trinitrate (GTN) or placebo. Error bars are one-sided standard deviations

Study II

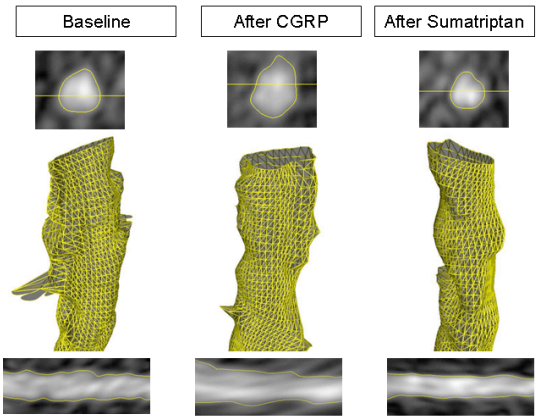
Dilatation by CGRP of middle meningeal artery and reversal by sumatriptan in normal volunteers

Effect of CGRP on MMA and MCA

We found a difference in the MMA circumference (T_{30}- T_{-10} changes) between CGRP, 0.91 mm ± 0.67, and placebo, 0.33 mm ± 0.67, *(P = 0.006)*. The MMA dilated by 9.2% (1.2% to 17.1%, CI) after CGRP and by 4.8% (-0.51% to 10.12%,CI) after placebo (Fig. 4

and fig. 5). After correction for placebo-induced dilatation the CGRP-induced dilatation amounted to 5.7% (1.2% to 10.1% CI). There was no change in the MCA circumference ($T_{30} - T_{10}$changes) between CGRP, -0.26 mm ± 0.82, and placebo, -0.50 mm ±2.17 *(P = 0.69)* (Fig. 4). The corresponding percent changes were 0.9% (-4.1% to 1.53%, CI) and 2.9% (-0.80%to 4.63%, CI) (Fig.6).

Figure 4:

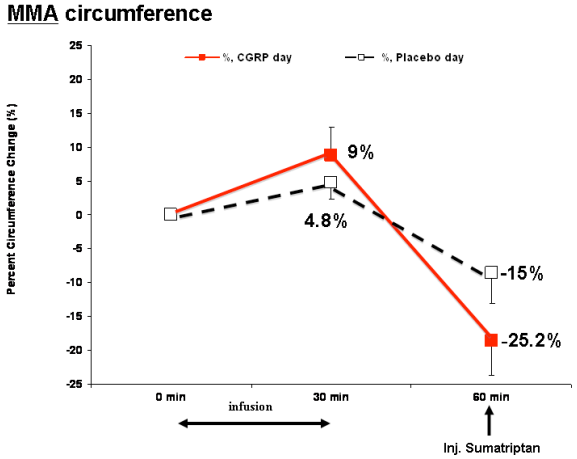


BOLD percent changes as function of measurement time relative to the time of start of injection. Acetazolamide significantly depressed BOLD responses at 5 min (P = 0.001), 25 min (P = 0.05) and 45 min (P = 0.003) compared to baseline. We found no statistical significant change in BOLD responses after glyceryl trinitrate (GTN) or placebo. Error bars are one-sided standard deviations

Effect of sumatriptan on MMA and MCA

Sumatriptan decreased both the MMA and the MCA circumference. Numerically, this response was greater on the MMA after CGRP than after placebo, but the difference was not significant. Thus, the MMA circumference tended to decrease more after CGRP, -1.27 mm ± 0.98, than after placebo, -0.83 mm ± 0.97, (absolute constriction: *P = 0.096*; the relative constriction: *P = 0.053*) (Fig. 5). Baseline corrected ratios between sumatriptan induced contraction and CGRP induced dilatation was -1.3 ± 0.5. The ratio between sumatriptan induced contraction and placebo induced dilatation was -5.8 ± 7.5. Paired sample T-test between the two ratios showed no difference *(P = 0.525)*.

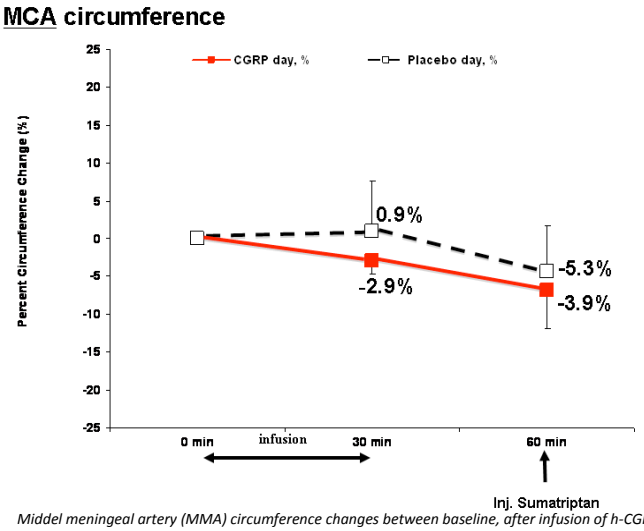
Figure 5:



Middel meningeal artery (MMA) circumference changes between baseline, after infusion of h-CGRP or placebo and after subcutaneous sumatriptan 6 mg in 17 healthy volunteers. Y axis shows relative (%) changes compared to baseline with the percent (%) values shown are relative changes between two measurements (baseline vs. infusion phase or infusion phase vs. after sumatriptan administration). Error bars indicate one-sided SEM. There was a significant 9% dilatation of MMA after CGRP compared to placebo (P = 0.006). Sumatriptan contracted the MMA by 25.2% (19.44% to 30.54% CI) on the CGRP day and by 15% (7.66% to 22.34% CI) on placebo day compared to the infusion phase.

MCA circumference (T_{60} - T_{-10}changes) did not differ between CGRP, -0.34 mm ± 0.49, and placebo, 0.05 mm ± 2.3, *(P = 0.44)* (Fig. 4). The MMA was contracted by 25.2% (19.44% to 30.54%, CI) and MCA by 3.9% (1.23% to 6.60%, CI) after sumatriptan on the CGRP day. On the placebo day, the MMA was contracted by 15% (7.66% to 22.34%, CI) and the MCA by 5.3% (2.34% to 8.27%, CI) (Fig. 5 & fig.6). Sumatriptan caused more contraction of the MMA than the MCA on both the CGRP *(P < 0.0001)* and placebo days *(P = 0.007)*.

Figure 6:

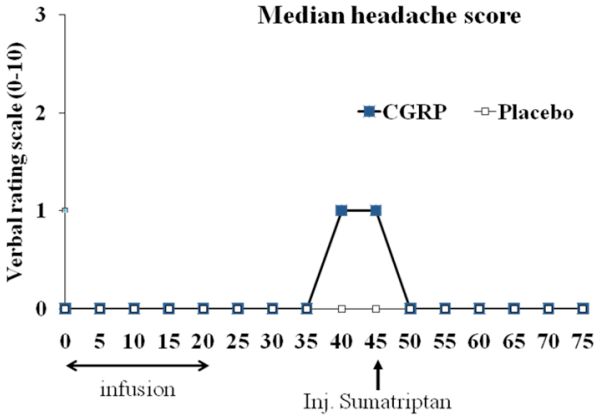


Middel meningeal artery (MMA) circumference changes between baseline, after infusion of h-CGRP or placebo and after subcutaneous sumatriptan 6 mg in 17 healthy volunteers. The Y axis shows relative (%) changes compared to baseline with the percent (%) values shown are relative changes between two measurements (baseline vs. infusion phase or infusion phase vs. after sumatriptan administration). Error bars indicate one-sided SEM. MCA circumference did not differ between CGRP and placebo (P > 0.05). Sumatriptan contracted MCA by 3.9% (1.23 to 6.60 % CI) on the CGRP day and by 5.3% (2.34% to 8.27% CI) after placebo compared to the infusion phase.

CGRP induced headache

The AUC for headache score (0 - 75 min) was larger on the CGRP day, 41.5 (17-54.5), than on the placebo day, 8.5 (0.0- 34.3), *(P = 0.003)* (Fig. 7). There was no difference in the AUC for headache scores after sumatriptan (45- 75 min) between the two study days *(P = 0.088)*. All participants were headache free at the end of the experiment (T_{75}).

Figure 7:



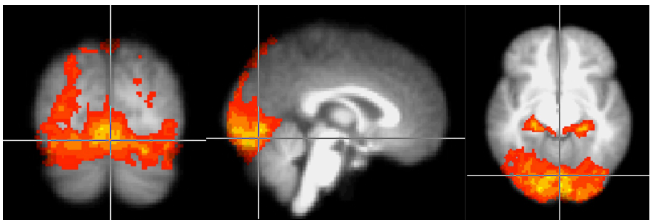
Median headache intensity on a verbal rating scale (0 – 10) during and after infusion of h-CGRP or placebo and after sumatriptan administration in 18 healthy volunteers. The peak headache-intensity occurred 40 min after start of infusion. 15 min after subcutaneous sumatriptan the median headache scores dropped to 0 (0-10 scale).

Study III

Pharmacological modulation of the BOLD response – a study of CGRP and sumatriptan in humans

All subjects showed a strong BOLD response to visual stimulation. Group activation is shown in Fig. 8. Table 1 shows vascular results.

Figure 8:

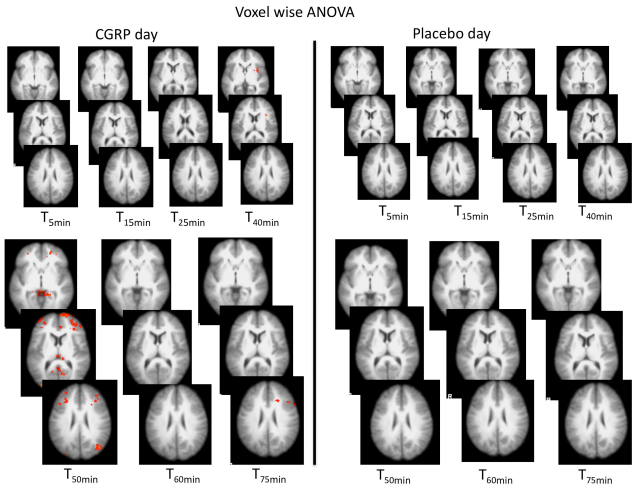


Group analysis of the baseline scans (before drug infusion) of 18 healthy subjects on CGRP study day. The images show a strong activation in the visual cortex after visual stimulation. Group analysis from the placebo day showed similar results.

The effect of CGRP on the BOLD response in the visual cortex

ANOVA did not show significant changes over time in activated voxels either on the CGRP day or on the placebo day (Fig. 9 and 10). ANOVA of the COPE recordings in the visual cortex revealed no statistical changes after CGRP *(P = 0.12)* or placebo infusion *(P = 0.41)*. We found no statistical difference with regard to activated voxels or recordings of COPE1-values between the two experimental days (*P = 0.357*) (Fig. 11).

Figure 9:

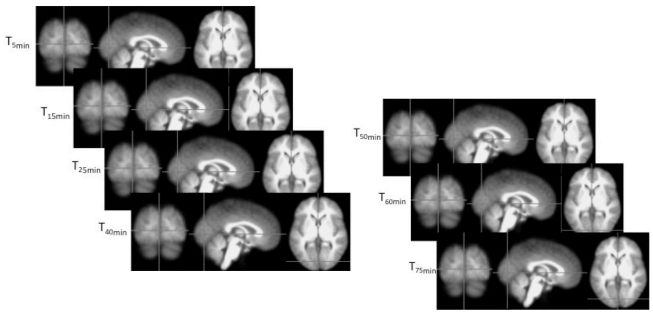


One way voxel-wise ANOVA results from the measured time points at the CGRP day (left side) and placebo day (right side). ANOVA showed no statistical changes over time in activated voxels after CGRP and placebo infusion except some scattered activation after sumatriptan injection on placebo day. The activated voxels were located primarily in the cerebellum and in the white matter of the corpus callosum. There were no activated voxels in the predefined visual region-of-interest .

The effect of sumatriptan on the BOLD response in the visual cortex

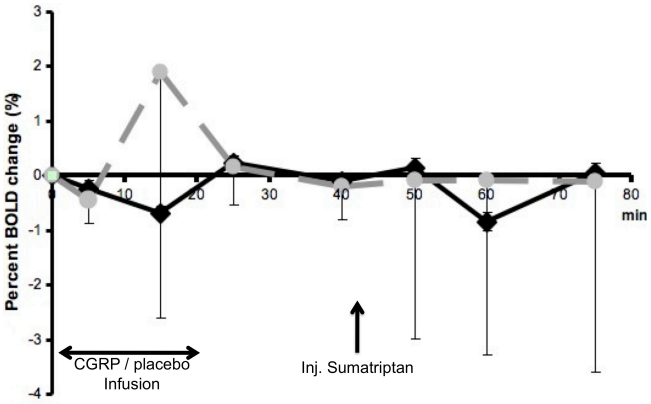
One-way voxel-wise ANOVA on the CGRP day showed scattered activation atT_{45min}. The activated voxels were located primarily in the cerebellum and in the white matter of the corpus callosum. There were no activated voxels in the visual cortex that was our predefined ROI or in other areas directly related to visual stimulation. The remaining recordings on the CGRP day did not show significantly activated voxels. One-way voxel-wise ANOVA on the placebo day revealed no significantly activated voxels (Fig 9 and 10). One way ANOVA of the COPE1 recording values revealed no statistical changes either on the CGRP day *(P =0.71)* or on the placebo day *(P = 0.98)*. We found no difference in activated voxels or recordings of COPE1 values between two experimental days *(P = 0.49)* (Fig. 11)

Figure 10:



Second level analysis revealed no statistical difference between two experimental days. The pictures show the mean subtracted values between placebo day and the CGRP day.

Figure 11:



Baseline corrected contrast of parameter estimate (COPE1) results from the visual region-of-interest. ANOVA revealed no statistical changes after CGRP (P = 0.12) or placebo infusion (P = 0.41). We found no statistical difference between two experimental days (P = 0.357). ANOVA revealed no statistical changes after injection of sumatriptan neither on the CGRP (P = 0.71) or placebo days (P = 0.98) . We found no statistical difference after sumatriptan between two experimental days (P = 0.49).

Tabel 1:

CGRP day					
	P_{CO2}	Blood pressure		Heart rate	O_2 saturation
		Systolic	Diastolic		
Baseline	5.21 (±0.072)	118.6 (±2.07)	66.93 (±1,83)	64.51 (±2.7)	98.62 (±0.30)
T_5min	5.22 (±0.073)	116.3 (±1.92)	66.94 (±1.97)	65.53 (±2.5)	98.73 (±0.33)
T_{10}min	5.33 (±0.084)	118.4 (±2.07)	66.88 (±1.76)	69.41 (±2.4)	98.67 (±0.31)
T_{20}min	5.18 (±0.083)	115.1 (±2.12)	63.94 (±1.84)	73.43 (±3.1)	98.83 (±0.42)
T_{40}min	5.16 (±0.084)	118.4 (±2.07)	66.88 (±1.76)	69.41 (±2.4)	98.67 (±0.33)
T_{50}min	5.17 (±0.078)	126.2 (±2.08)	79,18 (±1.82)	70.12 (±2.9)	98.67 (±0.31)
T_{70}min	5.22 (±0.083)	123.1 (±1.73)	76.03 (±1.74)	68.72 (±2.6)	98.57 (±0.43)
T_{75}min	5.16 (±0.072)	124.1 (±1.53)	75.06 (±1.68)	67.94 (±2.6)	98.57 (±0.37)

Placebo day					
	P_{CO2}	Blood pressure		Heart rate	O_2 saturation
		Systolic	Diastolic		
Baseline	5.21 (±0.085)	118.8 (±3.25)	67.88 (±2.17)	67.12 (±2.83)	98.50 (±0.27)
T_5min	5.24 (±0.099)	116.4 (±2.52)	67.65 (±2.09)	66.94 (±2.94)	98.63 (±0.38)
T_{10}min	5.21 (±0.082)	116.4 (±2.22)	66.69 (±1.55)	70.31 (±3.55)	98.57 (±0.43)
T_{20}min	5.26 (±0.090)	116.4 (±2.51)	69.29 (±2.21)	66.47 (±2.93)	98.63 (±0.38)
T_{40}min	5.26 (±0.082)	117.0 (±3.10)	69.71 (±2.30)	69.35 (±3.28)	98.50 (±0.38)
T_{50}min	5.24 (±0.080)	125.4 (±2.46)	82.12 (±2.15)	69.53 (±2.78)	98.75 (±0.16)
T_{70}min	5.19 (±0.080)	124.7 (±2.01)	78.12 (±2.28)	66.94 (±3.56)	98.75 (±0.25)
T_{75}min	5.21 (±0.063)	124.5 (±2.34)	76.41 (±2.24)	65.12 (±2.60)	98.87 (±0.13)

Mean (± SEM) end-tidal P_{CO2}, blood pressure, heart rate and O_2saturation during and after infusion of CGRP and Placebo.

DISCUSSION

Study I

Acetazolamides effect on cerebral hemodynamics

We found that acetazolamide increases CBF by 20-40%. This has been reproduced by SPECT [22], PET [42] and ASL fMRI studies [43]. The CBF increase is probably secondary to dilatation of the arterioles that in turn is induced by pH changes [44]. Acetazolamide's effect on CBV remains a subject of debate [45]. A SPECT study reported that acetazolamide induced a rapid and marked increase in CBF without changes in $CMRO_2$ and suggested no effect of acetazolamide on CBV [21]. However, two studies reported CBV changes in healthy volunteers after acetazolamide administration [42,45]. Yamauchi et al. performed PET and dynamic contrast enhanced susceptibility (DSC) MRI perfusion measurements in healthy subjects and found that MRI overestimated both CBF and CBV [45]. However, it is now generally agreed that reliable values of CBF and CBV are difficult to obtain with DSC MRI [46]. Okazawa et al. found a CBF and CBV increase following acetazolamide administration, but concluded that the CBV increase was probably due to dilatation of the large arteries [42]. The effect of acetazolamide on cerebral arteries is still unclear and a subject of debate.

Acetazolamides effect on the BOLD signal

We found that acetazolamide depressed the BOLD signal. We used a bolus dose of 1 g, which is sufficient enough to block CA [21]. We observed a maximum decrease in BOLD signal already 5 min after administration, which is a remarkable finding when keeping in mind that acetazolamide attains peak plasma levels 15 to 18 min after administration [47]. This suggests that a much smaller dose is probably sufficient to completely block CA. The halftime of I.V. acetazolamide is ~1.7 h [47]. This could explain why the BOLD response continued to be depressed during the observation period. It should be noted, however, that we found no statistical difference compared to baseline (before drug administration) or to placebo at the last 55min recording.

There is some controversy concerning the effect of acetazolamide on the BOLD signal. In support of the present results two previous human studies have reported a decrease in BOLD responses in healthy volunteers after a hand motor task (n=5) [43], and during a breath-holding task (n=17) [48]. A third study of anesthetized rats also has reported similar findings [49]. While two studies have reported an increased BOLD signal amplitude but a smaller BOLD response after acetazolamide during a hand motor task in four patients with unilateral occlusion of the internal carotid artery [50] and during a visual task in six healthy volunteers [51]. There are, however, some important methodological reservations concerning these studies. For instance the authors reported large inter-subject variability without presenting P values in the results section and both studies were not randomized nor placebo controlled.

The effect of CBF changes on the BOLD signal

Based on the present data we suggest that acetazolamide depressed the BOLD signal probably by increasing CBF. Our interpretation is supported by studies using different pharmacological substances to examine the relationship between the BOLD response and CBF. Studies reported that caffeine resulted in a lowering of CBF [52], and a subsequent increase in the BOLD response [52]. Alcohol increases global CBF [53] while causing a depression of the BOLD response [54]. Furthermore, Kruuse et al. reported that sildenafil neither changed CBF [55] nor BOLD response in healthy subjects [56]. Taken together, the previous and present data further add to the growing evidence of dependency between CBF and the elicited BOLD response, and suggest an inverse relationship.

The effect of GTN on cerebral hemodynamics

The vascular actions of the nitric oxide (NO) donor GTN include both a direct and an indirect vasodilatation. NO readily passes through the blood-brain-barrier and into the smooth muscle cells resulting in activation of cGMP, smooth muscle relaxation in the arteries and vasodilatation [57]. It seems that GTN does not change CBF [25, 39, 58, 59]. One study reported that infusion of GTN (0.5 mg/kg/min in 20 min) solely increased CBV by 13% without effecting CBF [25]. In this study CBV was measured by 99mTC labeled erythrocytes and CBF was measured by following the 133-Xenon brain washout curve after an abrupt halt of Xenon inhalation using SPECT. Notably, a rather low spatial resolution was employed (16 mm in the horizontal plane). Mean velocity measured by transcranial Doppler in the medial cerebral artery (MCA) showed a 20% decrease indicating a vasodilatation.

GTN's effect on the BOLD signal

Surprisingly we found no changes in BOLD response found after GTN administration. Given that GTN increases CBV, neural-activity dependent CBF increase on top of an already expanded CBV could hypothetically result in a higher BOLD response. Understanding this is therefore potentially important for understanding BOLD imaging.

Based on estimations of the micro-vascular morphometry it is believed that the majority of blood at baseline is partitioned in the venous compartment (46%) with the remaining being partitioned in the capillaries (33%) and arterial compartments(21%) [60]. According to animal studies the respective compartments are affected unequally by CBV changes induced by hypo- or hypercapnia and by somatosensory stimulation – where the majority of changes affects the venous compartment (36% to 62% depending on the sources) and only to a lesser extent the arterial and capillary compartments [61, 62]. Therefore given that (i) the GTN induced CBV changes primarily increase venous CBV and (ii) that ΔR2 is proportional to venous cerebral blood volume [63] an increased BOLD response during neural activation should be expected. However we observed no changes in BOLD response.

To explain this, the data can be interpreted in the framework of a widely used model for the relationship between the BOLD response on one side, and CBF, CBV and the cerebral metabolic rate of oxygen ($CMRO_2$) on the other side [53, 54]. Employing Fick's principle [64, 65] in understanding the concentration of venous deoxyhemoglobin to the transverse relaxation rate (R2*) we see that (i) resting flow (CBF) increases,(ii) flow change during stimulation ($CBF-CBF_0$), blood volume and metabolism parameters are constant while (iii) the BOLD response ($\Delta S/S_0$) (MR signal change upon stimulation (ΔS)/MR signal in resting (S_0)) decreases. This is in accordance with the acetazolamide results.

If resting blood volume (CBV_0) increases, while ($CBV-CBV_0$), flow and metabolism parameters are constant, then the BOLD response ($\Delta S/S_0$) is expected to increase. But this notion is not in

agreement with our GTN findings. These conclusions hold true even when both fractional blood flow (CBF/CBF_0) or blood volume (CBV/CBV_0) changes are assumed constant across pharmacological challenges, when absolute flow and volume changes are used. Furthermore, the assumption that $CBF_0$and CBV_0 are not coupled is not supported by previous studies. Instead the interrelationship between CBV and CBF has been confirmed in animal [62, 66]and human studies [67, 68]. Most notable is the relationship described by Grubb et al, who found that $CBV = 0.8 \times CBF^a$ (Where a was fund to be 0.38). According to Grubb et al. an isolated effect on CBV without a concomitant effect on CBF is simply not possible[69]. Therefore, we deduct that there is a mismatch between previous studies often based on nuclear medicine methods and our current understanding of the primary determinants of the BOLD response. When Grubbs principle is applied we again find that the acetazolamide results are in perfect accordance with what could be expected. But given that there is a coupling of blood flow and volume then a CBV_0 increase while ($CBV-CBV_0$) is constant should result in a decrease in BOLD response ($\Delta S/S$). The BOLD response is dependent on deoxygenation changesin the venoules and venous part of the capillaries [63]. One possible explanation for the lacking effect of GTN on the BOLD response could be that GTN affects the macrovascular part of the vascular system and does not have an effect on the microvascular system, which is involved in the BOLD response. Thus, we speculate that the lowering of the velocity in MCA after GTN suggest that large arteries dilate, and likely lengthen a bolus arrival time in a contrast based perfusion study.
However, this does not change the perfusion itself, because GTN is unlikely to dilate the resistance vessels. Furthermore, GTN may dilate large venous vessels, without affecting the venoules where passive inflation is caused by elevation of the venous blood pressure occurring secondary to a lowering of the arteriolar resistance. Concerning acetazolamide, it undoubtedly increases CBF and thereby inflates small venoules without effect on the large venous compartment.

STUDY II AND III

The effects of CGRP on extra cerebral arteries
We found that exogenous CGRP caused a modest dilatation (9%) of the MMA. It has previously been reported that infusion of CGRP (1.5 µg/min over 20 min)in man induced dilatation of the superficial temporal by 30% and 5% dilatation of the radial artery [32]. In arteries without the BBB exogenous CGRP diffuses through holes between the endothelial cells and reaches the smooth muscle of the artery. CGRP mediated relaxation is achieved by binding directly to CGRP receptors on the smooth muscle cell [70]. Previous in vitro studies of human arteries [70] and animal in vitro [71] and in vivo studies [72] have also reported dilatation of MMA.

The effect of CGRP on intra cerebral arteries
We found that CGRP did not dilate MCA. A previous study of Petersen et al. reported a modest dilatation (9% ± 8.1 SD) of MCA following CGRP infusion (1.5g/min over 20 min which is similar to ours) in healthy volunteers [32]. In a study, in migraine sufferers, Lassen et al. applied a higher dose of CGRP (2 g/min over 20 min) and reported dilatation of MCA by 7.5% [31]. The methodological reservation regarding these studies is that the data where obtained by means of indirect measurements. Transcranial Doppler (TCD) was used to measure blood velocity in MCA (VMCA) combined with global CBF measurements using SPECT. Thus Petersen et al. reported that CGRP had no effect on global or regional CBF [32]. Therefore, a minor drop of velocity in the MCA in TCD studies was interpreted as dilatation. But the authors them self's were unable to reproduce their results. In another study they reported significant dilatation between baseline and CGRP based on unchanged V_{MCA} in combination with increased global CBF (not CO_2 corrected), but found no difference between experimental days [32]. Lassen et al. reported a drop in V_{MCA} and unchanged CO_2-corrected regional CBF between baseline and after CGRP, which was interpreted as MCA dilatation. However, the authors found no difference between CGRP and placebo [31]. In the present study we utilized the MRA technique to perform direct measurement of the arterial circumference. The analysis was done blindly with respect to the experimental days and specialized software validated with regard to reproducibility was used for the analysis. In the analysis minimal user interference was required in order to diminish users bias [40]. The results of the present study are thus not based on interpretation and extrapolation from indirect measurements but are based on direct very precise measurements and are therefore in all likelihood true.

Is CGRP's vascular site-of-action intra- or extra cerebral?
The question is whether vascular responses of the intracranial vessels to systemic CGRP differ from that of extracranial vessels. It is highly likely that the lack of MCA dilatation is due to inability of CGRP to cross the blood brain barrier (BBB) and therefore exogenously administrated CGRP acts only in the extracerebral compartment. In support, the CGRP-receptor antagonist olcegepant (Boehringer Ingelheim Pharmaceuticals) had no effects on MCA velocity, but inhibited dilatation of extracerebral arteries such as STA and radial arteries [32, 33]. An in vivo animal study reported that olcegepant effectively inhibited CGRP-mediated vasodilatation of the rat MMA [72]. Interestingly, olcegepant did not block dilatation of pial arteries inducedby electrical stimulation. This type of dilatation is probably caused by abluminal release of CGRP from perivascular nerve Fibers and is unaffected by olcegepant [72]. Abluminally applied CGRP dilated rat MCA while CGRP given luminally did not [73]. Collectively, these data suggest that exogenous CGRP does not cross the BBB and does not dilate the MCA or other deep brain arteries with tight junctions.

Cerebral effect of CGRP
It has been suggested that CGRP, in addition to its strong vasodilatory effect, also acts as an important and widespread neuromodulator in the brain. To investigate this we used BOLD fMRI after visual checkerboard stimulation to study the effect of systemic CGRP on the brain. Checkerboard stimulation is a well validated, reproducible stimulation modality known to produce a large BOLD signal in the visual cortex [38]. The study showed no modulator effect of CGRP on the BOLD signal, despite the presence of headache.

CGRP is found in many regions of the CNS including visual cortex [74]. Interestingly, CGRP receptors are not detected in central glial cells or second order neurons [75]. It has been reported that CGRP facilitates glutamatergic neurotransmission in the dorsal horns of the spinal cord [76]. Furthermore, CGRP may modulate central sensitization by increasing the discharge frequency of wide dynamic range neurons in the spinal cord, thus modulating nociceptive transmission [77]. Interestingly, two studies have found that CGRP release is anti-nociceptive [78, 79].

An important question raised by CGRP provocation experiments is whether intravenous infusion of CGRP induces head pain inside or outside of the blood-brain barrier(BBB). The BBB is formed by the tight junctions between endothelial cells in cerebral vessels[80]. Given that the vascular smooth muscle cells are placed outside the BBB. Studies of cerebral arterial diameter or cerebral blood flow can therefore elucidate mechanisms underlying CGRP effects on the brain, when given systemically. Because CGRP failed to dilate the MCA or to modulate the BOLD signal this further supports the hypothesis that CGRP exerts its headache provoking action outside of the BBB.

The effect of Sumatriptan on intracraniel arteries

Sumatriptan (5-HT1B/1D receptor agonist) was originally developed as a selectivecranial vasoconstrictor [35] and this is still the most well documented mode of action of the drug . The vascular site-of-action of sumatriptan and other triptans in migraine has been a sub-ject of debate. We found that sumatriptan reduced MMA circumference after CGRP pretreatment by 25%.As expected, sumatriptan also constricted MMA (15%) on the placebo day. Ratio calculations revealed that there was no statistical difference in the constrictor capabilities of sumatriptan between the two days. On both experimental days we observed constriction (CGRP day: 3.9%; Placebo day: 5.3%) of the MCA. The effect was considerably larger on MMA than on MCA. These data suggests that sumatriptan exerts part of its anti-nociceptive action by constricting MMA and less so the MCA.

The effect of sumatriptan on CGRP induced headache

We found that the maximal median headache intensity occurred 40 min after start of CGRP infusion. Previous [31, 33] and present data suggest the maximum vasodilator response 30-90 min after start of infusion. Sumatriptan was administered subcutaneously 45 min after start ofthe CGRP/placebo infusion. The vascular responses were recorded 15 min after sumatriptan administration. Although sumatriptan reversed CGRP induced MMA dilatation, the AUC for headache scores after sumatriptan was reduced but did not reach statistical difference compared to placebo. There could be several explanations for these observations. First, the study was powered to examine effects on vascular responses and not to detect a correlation between headache and vascular effects. Second, on both study days sumatriptan caused commonly reported adverse events such as transient worsening of headache [81] which could influence results. Third, the CGRP induced immediate headache was very mild in our normal volunteers (maximum median intensity 1 on VRS) and short lasting, why a possible anti-nociceptive effect would therefore be difficult to detect. Finally, 15 min is a very short time even for subcutaneous sumatriptan [82]. Future studies should examine the effect of sumatriptan during CGRP induced delayed migraine like attacks in migraineurs.

Effect of sumatriptan on brain activity

The mechanism behind anti-migraine action of triptans remains a matter of intense research and debate [35, 83]and is more complex then initially perceived. Triptans lower the CGRP concentration in animals [84, 85] and in nitric oxide induced [86]and spontaneous migraine attacks in humans [87]. The anti-migraine effect of the triptans might therefore be due to inhibited release of CGRP [88, 89]. This effect could be mediated via 5HT1B/D-receptors, because antagonists to this receptor block the CGRP attenuating effect of sumatriptan [90, 91]. Interestingly, an in-vitro study in cultured trigeminal neurons reported that sumatriptan decreased CGRP secretion from electrically or chemically stimulated sensory neurons, but not the basal secretion rate [85]. A recent study in healthy volunteers confirmed that sumatriptan did not affect CGRP levels under baseline conditions [92].

Electrophysiological studies on animals have showed peripheral [93]and central sites of action in the trigeminal pain pathway [94].Sakai et al. reported that the increased brain 5-HT synthetic rate during a migraine attack was reversed by sumatriptan [95]. It is, however, unclear whether this was due to a direct effect of sumatriptan on brain activity. We recorded neuronal activity by BOLD-fMRI and possible arterial dilatation by MRA simultaneously. We found that sumatriptan failed to affect brain activity.
We thus found that sumatriptan contracted MMA but failed to affect brain activity and only had a marginal effect on MCA. In a recent study of migraine without aura induced by CGRP, sumatriptan only contracted MMA and failed to induce MCA contraction [96]. Taken together this further suggests that sumatriptan primarily acts outside the BBB and mimics its effects by contracting the MMA.

METHODOLOGICAL CONSIDERATIONS

In study I we did not measure the effect of acetazolamide and GTN on CBF and CBV. One previous study has measured CBV using nuclear imaging technique [25]. We would have preferred to have measured hemodynamic variables simultaneously with obtaining BOLD fMRI images. This would both have verified the information derived from previous studies and further strengthen our argumentation by making it possible to construct a model of the interrelationship between BOLD signal and hemodynamic variables. Using MRI to perform quantitative measurement of CBF, CBV and $CMRO_2$ though remains a challenge.

In study II we measured the initial part of the MMA. It is possible that we would have recorded a larger dilatation if we also measured changes in the intracranial part of MMA. We used a novel high resolution MRA necessary for discovering sudden dynamic changes in the arteries. Performing MRA's on a larger segment of the arteries would have increased the scan duration significantly – beyond what would be practically feasible. In addition, the high-resolution images served to limit partial-volume related problems. For the statistical calculations we used the averaged circumference data derived from measurements preformed every 0.2mm along the length of the arteries. We preferred circumference measurements over diameter measurements because the software calculates the diameter based on the assumption that arteries act as perfect circles. The circumference is instead measured directly, and in addition gives more precise results because it is taken into account that the brain arteries are not uniform and does not resemble perfect circles.

In study III we used visual stimulation as a surrogate maker for the global effect of CGRP on the brain activity. One might argue that it would have been more relevant to use sensory stimulation to study the trigemino-pain pathways. Visual stimulation was chosen because (i) it produces a large BOLD signal, which makes it easier to quantify [38]. (ii) According to existing literature CGRP receptors are evenly distributed in the brain cortex [74,97] and therefore exogenous CGRP should affect the brain evenly. Lastly the scope of this study was to determine if CGRP has a modulator

effect on brain activity per see. In principle, all headache subtypes including pharmacologically induced headaches are associated with activation of central pain pathways. Incase a ROI in the trigeminal pain pathway was chosen directly and if the results showed increased activity in that particular ROI it would remain uncertain if the increased activity was due to the direct effect of CGRP per see or because of increased activity due to the induced headache.

CONCLUSION AND FUTURE PERSPECTIVES

MRI and fMRI provide important information on mechanisms and site-of-action of pharmacological substances. In addition, pharmacological agents can be used as a tool in investigation of brain physiology. BOLD fMRI is a non-invasive method that measures neuronal activity indirectly. Changes in brain hemodynamics such as CBF, CMRO 2 and CBV can alter the measured BOLD signal[8]. Therefore, investigation of the direct effect of pharmacological substances on the BOLD response in normal or baseline conditions is crucial before the method can be employed for in vivo localization of activity or used to study disease and pathophysiology. MRA can be implored to examine possible dynamic changes in the brain arteries thus reflecting possible vascular effects of a pharmacological compound.

The study I showed that acetazolamide depresses the BOLD signal and thus adds to the growing evidence of the inverse relationship between CBF and BOLD signal. The NO donor GTN is known to increase CBV. Contrary to our expectations this did not result in increased BOLD signal. Based on the model offered in this thesis the most possible explanation is that the GTN induced CBV increase is anatomically located primarily in the large arteries. While the BOLD measurements are based on recording of changes in the microvascular compartment i.g.in the capillaries and venoules.

The study II and III showed that CGRP infusion caused headache and dilatation of MMA, while MCA and brain activity remained unchanged. This indicates that CGRP does not cross the BBB and that capillaries, venoules and arteries inside the BBB remains unaffected by CGRP. Therefore, it seems that CGRP induced immediate headache is caused by dilatation of meningeal arteries combined with release of signaling molecules from the perivascular nerve endings. The selective intracranial vasoconstrictor sumatriptan (5-HT1B/1D receptor agonist) contracts MMA and reverses CGRP induced dilatation, while sumatriptan only marginally contracts MCA and has no effect on the BOLD signal. We furthermore found that sumatriptan had a more selective action on MMA compared to MCA. This indicates that sumatriptan exerts its effect outside of BBB. This suggests that dilatation of MMA is important for experimentally provoking neurovascular headache, while contraction of MMA could be an important potential target for development of future drugs against neurovascular headache. While intracerebral arteries seems to remain passive when treatment against headache is administered.

This thesis shows that phMRI is a useful tool in understanding mechanisms and site-of-action of pharmacological compounds. phMRI could be a beneficial addition to the existing methods for development of future drugs. The present data in addition underline the importance of further exploration of methodological aspects concerning the BOLD response. This thesis underlines the importance of addressing how changes in brain hemodynamic may modulate the BOLD signal in order to avoid false negative or false positive results. Especially in case hemodynamic changes are introduced by pharmacological perturbation the effect of the perturbation on the BOLD response per se must be characterized before the data can be interpreted as changes in neuronal activity. In addition, this thesis has shown that brain arteries are complex heterogeneous structures with varying function according to location and anatomy. The arteries are involved in regulating brain homeostasis and could therefore qualify as important future drug targets.

SUMMARY

Over the last decades MRI has proved to be very useful in the field of drug development and drug discovery. Pharmacological MRI (phMRI) explores the interaction between brain physiology, neuronal activity and drugs. The BOLD signal isa n indirect method to investigate brain activity by way of measuring task related hemodynamic changes. Pharmacological substances that induce hemodynamic changes can therefore potentially alter the BOLD signal and in turn falsely can be interpreted as changes in neuronal activity. It is therefore important to characterize possible effects of a pharmacological substance on the BOLD response per see before that substance can be used in an fMRI experimental setup in order to avoid false positive or false negative results. Furthermore MRI and MRA is useful in determining the vascular site-of-action of vasoactive sub-stances. Four substances; acetazolamide, GTN, CGRP and sumatriptan has been examined in double-blind placebo controlled crossover studies.

The present thesis includes three papers with the aim to determine the site-of-action and to explore the effects of the pharmacological agents on the BOLD signal.

Study I showed that acetazolamide depressed the BOLD signal by increasing CBF.GTN is known to increase CBV but had surprisingly no effect on the BOLD signal. This is probably because the GTN induces CBV increase is limited to the large arteries whereas the hemodynamic changes associated with the BOLD signal are anatomically located in the capillaries and venoules. Study II and III showed that systemic administration of CGRP induces immediate headache and dilates the MMA but contrarily to previous belief does not dilate the MCA. Nor does CGRP increase brain activity per see. Sumatriptan ameliorates headache, contracts MMA and marginally contrasts MCA without altering brain activity.

In conclusion we found that acetazolamide depresses the BOLD signal while GTN does not alter the BOLD signal. While systemic administration of CGRP or sumatriptan has no direct effects on brain activity in healthy volunteers. Instead it seems that both migraine provoking peptide CGRP and anti-migraine drug sumatriptan exert their actions outside of the BBB.

This thesis shows that phMRI is a powerful tool in understanding mechanisms and site-of-action of pharmacological compounds. phMRI could be a useful addition to the existing methods for development of future drugs

LIST of ABBREVIATIONS

AE	Adverse events
ASL	Arterial spin labeling
ANOVA	Analysis of Variance
AUC	Area under the curve
BBB	Blood brain barrier
BET	Brain extraction tool
BOLD	Blood oxygenation level dependent
CA	Carbon anhydrase
CAI	Carbon anhydrase inhibitor
CAT	Computer attenuated tomography
CBF	Cerebral blood flow
CBV	Cerebral blood volume
CGRP	Calcitonin gene-related peptide
CI	Confidence interval
CMRO2	Cerebral metabolic rate of oxygen
COPE	Contrast of parameter estimates
FDR	False discovery rate
fMRI	Functional Magnetic resonance imaging
FMRIB	Oxford Centre for Functional Magnetic Resonance Imaging of the Brain
FOV	Field of view
FSL	FMRIB Software Library
GLM	General linear model
GTN	Glyceryl trinitrate
HR	Heart rate
IHS	International headache society
MCA	Middle cerebral artery
MMA	Middle meningeal artery
MNI-152	Montreal neurological institute
MRI	Magnetic resonance imaging
MO	Migraine without aura
NO	Nitrogen oxide
PaO2	Arterial partial pressure of O2
PCO2	Partial pressure of CO2
PET	Positron emission tomography
phMRI	Pharmacological magnetic resonance imaging
QA	Quality assurance
RF	Radio frequency
ROI	Region-of-interest
DS	MR signal change upon stimulation
S0	MR signal at rest
SD	Standard deviation
SEM	Standard error of the mean
SPECT	Single photon emission computed tomography
STA	Superficial temporal artery
TCD	Transcranial Doppler
TE	Echo time
TR	Repetition time
VAS	Visual analogue scale
VMCA	Velocity of the middle cerebral artery
VRS	Verbal rating scale
5-HT	5-hydroxytryptamine

REFERENCES

1. Huettel S., Song AW. & McCarthy G., 2004. Functional magnetic resonance imaging, ISBN 0-87893-288-7,Sunderland Mass.: Sinauer Associates Publishers.
2. Jezzard P., Matthews P. & Smith S., Worsley KJ., Statistical analysis of activation images. Ch 14. In Functional MRI: An Introduction to Methods. ISBN 0-19-263071-1. New York, NY: Oxford University Press.
3. Shenton ME. et al., 2001. A review of MRI findings in schizophrenia. Schizophrenia Research, 49(1-2), pp.1-52.
4. Leslie RA. & James MF., 2000. Pharmacological magnetic resonance imaging: a new application for
5. functional MRI. Trends in Pharma. Sciences, 21(8), pp.314-318.
6. Kwong K., 1995. Functional magnetic resonance imaging with echo planar imaging. MagnReson.Q.,11(1), pp.1-20.
7. Ogawa, S. et al., 1990. Oxygenation-sensitive contrast in magnetic resonance image of rodent brain at high magnetic fields. MagnReson.Med., 14(1), pp.68-78.
8. Kida I., Rothman D. & Hyder F., 2007. Dynamics of changes in blood flow, volume, and oxygenation: implications for dynamic functional magnetic resonance imaging calibration. J.Cereb.Blood Flow Metab, 27(4), pp.690-696.
9. Buxton R., Wong E. & Frank L., 1998. Dynamics of blood flow and oxygenation changes during brain activation: the balloon model. Magn Reson.Med., 39(6), pp.855-864.
10. Logothetis N. &Wandell B., 2004. Interpreting the BOLD signal. Annu.Rev.Physiol, 66, pp.735-769.
11. Graves M., 1997. Magnetic resonance angiography. Br J Radiol, 70(829), pp.6-28.
12. Toga A. &Mazziotta J., 2002. Brain Mapping: The Methods, Second Edition (Toga, Brain Mapping), AcademicPress.
13. Strandgaard S. et al., 1973. Autoregulation of Brain Circulation in Severe Arterial Hypertension. British Medical Journal, 1(5852), pp.507 -510.
14. Wise R. & Tracey I., 2006. The role of fMRI in drug discovery. J.MagnReson.Imaging, 23(6), pp.862-876.
15. Logothetis N. et al., 2001. Neurophysiological investigation of the basis of the fMRI signal. Nature, 412(6843), pp.150-157.
16. Buxton R. & Frank L., 1997. A model for the coupling between cerebral blood flow and oxygen metabolism during neural stimulation. J.Cereb.Blood Flow Metab, 17(1), pp.64-72.
17. Kwong K. et al., 1992. Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. Proc.Natl.Acad.Sci.U.S.A, 89(12), pp.5675-5679.
18. Busija DW. et al., 2008. Mechanisms involved in the cerebrovascular dilator effects of cortical spreading depression. Progress in Neurobiology, 86(4), pp.379-395.
19. May A. &Goadsby PJ., 1999. The Trigeminovascular System in Humans: Pathophysiologic Implications for Primary Headache Syndromes of the Neural Influences on the Cerebral Circulation. J.Cereb. Blood Flow Metab, 19(2), pp.115-127.
20. Thomsen LL. &Olesen J., 1995. The autonomic nervous system and the regulation of arterial tone in migraine. Clinical Autonomic Research, 5(5), pp.243-250.
21. Sorteberg W. et al., 1989. Effect of acetazolamide on cerebral artery blood velocity and regional cerebral blood flow in normal subjects. ActaNeurochir.(Wien.), 97(3-4), pp.139-145.
22. Vorstrup S., Henriksen L. & Paulson O., 1984. Effect of acetazolamide on cerebral blood flow and cerebral metabolic rate for oxygen. J.Clin.Invest, 74(5), pp.1634-1639.
23. Bonte F., Devous M. & Reisch J., 1988. The effect of acetazolamide on regional cerebral blood flow in normal human subjects as measured by single-photon emission computed tomography. Invest Radiol., 23(8), pp.564-568.
24. Hansen J. et al., 2007. Magnetic resonance angiography shows dilatation of the middle cerebral artery after infusion of glyceryltrinitrate in healthy volunteers. Cephalalgia, 27(2), pp.118-127.
25. Schoonman G. et al., 2006. Magnetic resonance angiography of the human middle meningeal artery: implications for migraine. J.MagnReson.Imaging, 24(4), pp.918-921.
26. Iversen H. et al., 2008. Intracranial hemodynamics during intravenous infusion of glyceryl trinitrate. J.Headache Pain, 9(3), pp.177-180.
27. Uddman R. et al., 1986. Calcitonin gene-related peptide (CGRP): perivascular distribution and vasodilatory effects. Regulatory Peptides, 15(1), pp.1-23.
28. Arulmani U. et al., 2004. Calcitonin gene-related peptide and its role in migraine pathophysiology. Eur.J.Pharmacol., 500(1-3), pp.315-330.
29. Brain SD. et al., 1985. Calcitonin gene-related peptide is a potent vasodilator. Nature, 313(5997), pp.54-56.
30. Brain S. & Cambridge H., 1996. Calcitonin gene-related peptide: vasoactive effects and potential therapeutic role. Gen.Pharmacol., 27(4), pp.607-611.
31. Wimalawansa SJ., 1996. Calcitonin gene-related peptide and its receptors: molecular genetics, physiology, pathophysiolo-

gy, and therapeutic potentials. Endocrine Reviews, 17(5), pp.533-585.
32. Lassen LH. et al., 2008. Involvement of calcitonin gene-related peptide in migraine: regional cerebral blood flow and blood flow velocity in migraine patients. J. of Headache and Pain, 9(3), pp.151-157.
33. Petersen K., Birk S. et al., 2005. The CGRP-antagonist, BIBN4096BS does not affect cerebral or systemic haemodynamics in healthy volunteers. Cephalalgia, 25(2), pp.139-147.
34. Petersen K., Nilsson E. et al., 2005. Presence and function of the calcitonin gene-related peptide receptor on rat pial arteries investigated in vitro and in vivo. Cephalalgia, 25(6), pp.424-432.
35. Lassen L. et al., 2002. CGRP may play a causative role in migraine. Cephalalgia, 22(1), pp.54-61.
36. Humphrey PP. &Goadsby PJ., 1994. The mode of action of sumatriptan is vascular? A debate. Cephalalgia:, 14(6), pp.401-410; discussion 393.
37. Friberg L. et al., 1991. Migraine pain associated with middle cerebral artery dilatation: reversal by sumatriptan. Lancet, 338(8758), pp.13-17.
38. Belliveau, J. et al., 1992. Magnetic resonance imaging mapping of brain function. Human visual cortex. Invest Radiol., 27 Suppl 2, pp.S59-S65.
39. Genovese C., Lazar N. & Nichols T., 2002. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. Neuroimage., 15(4), pp.870-878.
40. Iversen H., Olesen J. & Tfelt-Hansen P., 1989. Intravenous nitroglycerin as an experimental model of vascular headache. Basic characteristics. Pain, 38(1), pp.17-24.
41. deKoning P. et al., 2003. Automated segmentation and analysis of vascular structures in magnetic resonance angiographic images. MagnReson.Med., 50(6), pp.1189-1198.
42. Matthews J. et al., 1990. Analysis of serial measurements in medical research. BMJ, 300(6719), pp.230-235.
43. Okazawa H. et al., 2001. Effects of acetazolamide on cerebral blood flow, blood volume, and oxygen metabolism: a positron emission tomography study with healthy volunteers. J.Cereb.Blood Flow Metab, 21(12), pp.1472-1479.
44. Brown G. et al., 2003. BOLD and perfusion response to finger-thumb apposition after acetazolamide administration: differential relationship to global perfusion. J.Cereb.Blood Flow Metab, 23(7), pp.829- 837.
45. Travis DM. et al., 1964. Selective renal cardonic anhydrase inhibition without respiratory effect: Pharmacology of 2-Benzenesulfonamido-1,3, 4-Thiadiazole-5sulfonamide (CL 11,366). J. Pharma. Experimemtal Therapeutics, 143, pp.383-394.
46. Grandin CB. et al., 2005. Absolute CBF and CBV measurements by MRI bolus tracking before and after acetazolamide challenge: repeatabilily and comparison with PET in humans. NeuroImage, 26(2), pp.525-535.
47. Kiselev VG., 2005. Transverse relaxation effect of MRI contrast agents: a crucial issue for quantitative measurements of cerebral perfusion. JMRI, 22(6), pp.693-696.
48. Maren TH & Robinson B., 1960. The pharmacology of acetazolamide as related to cerebrospinal fluid and the treatment of hydrocephalus. Bulletin of the Johns Hopkins Hospital, 106, pp.1-24.
49. Shiino A. et al., 2003. Estimation of cerebral perfusion reserve by blood oxygenation level-dependent imaging: comparison with single-photon emission computed tomography. J.Cereb.Blood Flow Metab, 23(1), pp.121-135.
50. Mukherjee B. et al., 2005. Mapping of the cerebral response to acetazolamide using graded asymmetric spin echo EPI. MagnReson.Imaging, 23(9), pp.907-920.
51. Kleinschmidt A. et al., 1995. Magnetic resonance imaging of regional cerebral blood oxygenation changes under acetazolamide in carotid occlusive disease. Stroke, 26(1), pp.106-110.
52. Bruhn, H. et al., 1994. The effect of acetazolamide on regional cerebral blood oxygenation at rest and under stimulation as assessed by MRI. J.Cereb.Blood Flow Metab, 14(5), pp.742-748.
53. Mulderink T. et al., 2002. On the use of caffeine as a contrast booster for BOLD fMRI studies. Neuroimage., 15(1), pp.37-44.
54. Newlin D. et al., 1982. Effect of alcohol ingestion on regional cerebral blood flow. Int.J.Neurosci., 17(3), pp.145-150.
55. Levin J. et al., 1998. Reduction in BOLD fMRI response to primary visual stimulation following alcohol ingestion. Psychiatry Res., 82(3), pp.135-146.
56. Kruuse C. et al., 2002. The phosphodiesterase 5 inhibitor sildenafil has no effect on cerebral blood flow or blood velocity, but nevertheless induces headache in healthy subjects. J.Cereb.Blood Flow Metab, 22(9), pp.1124-1131.
57. Kruuse C. et al., 2009. Cerebral haemodynamic response or excitability is not affected by sildenafil. J.Cereb.Blood Flow Metab. Available at: PM:19209179.
58. Archer SL. et al., 1994. Nitric oxide and cGMP cause vasorelaxation by activation of a charybdotoxinsensitive K channel by cGMP-dependent protein kinase. Proc.Natl.Acad.Sci.U.S.A, 91(16), pp.7583- 7587.
59. Iversen HK. et al., 1990. Arterial responses during migraine headache. Lancet, 336(8719), pp.837-839.
60. White RP. et al., 2000. The effect of the nitric oxide donor glyceryltrinitrate on global and regional cerebral blood flow in man. J. Neuro. Sciences, 178(1), pp.23-28.
61. vanZijl PC. et al., 1998. Quantitative assessment of blood flow, blood volume and blood oxygenation effects in functional magnetic resonance imaging. Nature Medicine, 4(2), pp.159-167.
62. Mandeville JB. et al., 1999. Evidence of a cerebrovascular postarteriolewindkessel with delayed compliance. J.Cereb.Blood Flow Metab, 19(6), pp.679-689.
63. Lee SP. et al., 2001. Relative changes of cerebral arterial and venous blood volumes during increased cerebral blood flow: implications for BOLD fMRI. MagnReson.Med, 45(5), pp.791-800.
64. Cohen ER. et al., 2004. Hypercapnic normalization of BOLD fMRI: comparison across field strengths and pulse sequences. NeuroImage, 23(2), pp.613-624.
65. Davis T. et al., 1998. Calibrated functional MRI: mapping the dynamics of oxidative metabolism. Proc. Natl.Acad.Sci.U.S.A, 95(4), pp.1834-1839.
66. Hoge R. et al., 1999. Investigation of BOLD signal dependence on cerebral blood flow and oxygen consumption: the deoxyhemoglobin dilution model. MagnReson.Med., 42(5), pp.849-863.
67. Wu G. et al., 2002. Transient relationships among BOLD, CBV, and CBF changes in rat brain as detected by functional MRI. MagnReson.Med., 48(6), pp.987-993.
68. Chen JJ. & Pike GB., 2009. BOLD-specific cerebral blood volume and blood flow changes during neuronal activation in humans. NMR in Biomedicine, 22(10), pp.1054-1062.

69. Rostrup E. et al., 2005. The relationship between cerebral blood flow and volume in humans. Neuroimage., 24(1), pp.1-11.
70. Grubb R. et al., 1974. The effects of changes in PaCO2 on cerebral blood volume, blood flow, and vascular mean transit time. Stroke, 5(5), pp.630-639.
71. Jansen-Olesen I. et al., 2003. In-depth characterization of CGRP receptors in human intracranial arteries. Euro. J. of Pharmacology, 481(2-3), pp.207-216.
72. Grubb R. et al., 1974. The effects of changes in PaCO2 on cerebral blood volume, blood flow, and vascular mean transit time. Stroke, 5(5), pp.630-639.
73. Petersen K. et al., 2004. Inhibitory effect of BIBN4096BS on cephalic vasodilatation induced by CGRP or transcranial electrical stimulation in the rat. Br.J.Pharmacol., 143(6), pp.697-704.
74. Petersen KA. et al., 2005. BIBN4096BS antagonizes human alpha-calcitonin gene related peptide induced headache and extracerebral artery dilatation. Clinical Pharmacology and Therapeutics, 77(3), pp.202-213.
75. Ho TW.,Edvinsson L. &Goadsby PJ., 2010. CGRP and its receptors provide new insights into migraine pathophysiology. Nature Reviews. Neurology, 6(10), pp.573-582.
76. Lennerz JK. et al., 2008. Calcitonin receptor-like receptor (CLR), receptor activity-modifying protein 1 (RAMP1), and calcitonin gene-related peptide (CGRP) immunoreactivity in the rat trigeminovascular system: differences between peripheral and central CGRP receptor distribution. J. of Comparative Neurol., 507(3), pp.1277-1299.
77. Gu X. & Yu L., 2007. The colocalization of CGRP receptor and AMPA receptor in the spinal dorsal horn neuron of rat: a morphological and electrophysiological study. Neuroscience Letters, 414(3), pp.237-241.
78. Yu Y., Lundeberg T. & Yu LC., 2002. Role of calcitonin gene-related peptide and its antagonist on the evoked discharge frequency of wide dynamic range neurons in the dorsal horn of the spinal cord in rats. Regulatory Peptides, 103(1), pp.23-27.
79. Pecile A. et al., 1987. Calcitonin gene-related peptide: antinociceptive activity in rats, comparison with calcitonin. Regulatory Peptides, 18(3-4), pp.189-199.
80. Huang Y. et al., 2000. Anti-nociceptive effects of calcitonin gene-related peptide in nucleus raphe magnus of rats: an effect attenuated by naloxone. Brain Research, 873(1), pp.54-59.
81. Wolburg H. &Lippoldt A., 2002. Tight junctions of the blood-brain barrier: development, composition and regulation. Vascular Pharmacology, 38(6), pp.323-337.
82. Ferrari MD. et al., 2002. Triptans (serotonin, 5-HT1B/1D agonists) in migraine: detailed results and methods of a meta-analysis of 53 trials. Cephalalgia:, 22(8), pp.633-658.
83. Lacey LF, Hussey EK, Fowler PA., 1995. Single dose pharmacokinetics of sumatriptan in healthy volunteers. Eur J ClinPharmacol. 47(6):543-8.
84. Ahn A. &Basbaum A., 2005. Where do triptans act in the treatment of migraine? Pain, 115(1-2), pp.1-4.
85. Buzzi MG. et al., 1991. Dihydroergotamine and sumatriptan attenuate levels of CGRP in plasma in rat superior sagittal sinus during electrical stimulation of the trigeminal ganglion. Neuropharmacology, 30(11), pp.1193-1200.
86. Eltorp CT., Jansen-Olesen I. & Hansen AJ., 2000. Release of calcitonin gene-related peptide (CGRP) from guinea pig dura mater in vitro is inhibited by sumatriptan but unaffected by nitric oxide. Cephalalgia: 20(9), pp.838-844.
87. Juhasz G. et al., 2005. Sumatriptan causes parallel decrease in plasma calcitonin gene-related peptide (CGRP) concentration and migraine headache during nitroglycerin induced migraine attack. Cephalalgia, 25(3), pp.179-183.
88. Sarchielli P. et al., 2000. Nitric oxide metabolites, prostaglandins and trigeminal vasoactive peptides in internal jugular vein blood during spontaneous migraine attacks. Cephalalgia:, 20(10), pp.907-918.
89. Durham .L. & Russo AF., 1999. Regulation of calcitonin gene-related peptide secretion by a serotonergic antimigraine drug. J. of Neuroscience, 19(9), pp.3423-3429.
90. Durham P., 2008. Inhibition of calcitonin gene-related peptide function: a promising strategy for treating migraine. Headache, 48(8), pp.1269-1275.
91. Cumberbatch M. et al., 1999. Dural vasodilation causes a sensitization of rat caudal trigeminal neurons in vivo that is blocked by a 5-HT1B/1D agonist. Br.J.Pharmacol., 126(6), pp.1478-1486.
92. Ferrari M. &Saxena P., 1993. Clinical and experimental effects of sumatriptan in humans. Trends Pharmacol. Sci., 14(4), pp.129-133.
93. Hansen J. et al., 2009. Sumatriptan does not change calcitonin gene-related peptide in the cephalic and extracephalic circulation in healthy volunteers. J.Headache Pain, 10(2), pp.85-91.
94. Levy D., Burstein R. & Strassman A., 2005. Calcitonin gene-related peptide does not excite or sensitize meningeal nociceptors: implications for the pathophysiology of migraine. Ann.Neurol., 58(5), pp.698-705.
95. Afridi SK. et al., 2005. A PET study exploring the laterality of brainstem activation in migraine using glyceryltrinitrate. Brain, 128(Pt 4), pp.932-939.
96. Sakai Y. et al., 2008. Sumatriptan normalizes the migraine attack-related increase in brain serotonin synthesis. Neurology, 70(6), pp.431-439.
97. Asghar MA. et al. 2011., Evidence for a vascular factor in migraine. Ann. of Neurol., 69(4):635-45
98. vanRossum D, Hanisch UK, Quirion R., 1997. Neuroanatomical localization, pharmacological characterization and functions of CGRP, related peptides and their receptors.NeurosciBiobehav Rev. Sep;21(5):649-78.
99. Borsook D., Becerra L. & Hargreaves R., 2006. A role for fMRI in optimizing CNS drug development. Nat.rev.Drugdiscov., 5(5), pp.411-424.