Title in the application: Summary of the study EudratCT 2007-004250-81 (AKREMITOLFMRI1): Acute opioid tolerance as revealed by functional magnetic resonance imaging

The analysis of the imaging results has taken a very long time as this was a very challenging study to perform. It is expected that the final manuscript will be submitted this spring. Here, we present the a summary of the results.

Introduction

The effects of opioids in humans include analgesia, sedation, euphoria or dysphoria, and respiratory depression. Development of tolerance after long-term opioid administration of opioids is well known whereas acute tolerance has been less studied. It has been shown in rats after a single-dose of opioid (Larcher *et al*, 1998). Acute opiate tolerance has also been reported in humans (McQuay *et al*, 1981).

In the current study, we used functional magnetic resonance imaging (fMRI) and BOLD signal to investigate the effect of remifentanil and placebo boluses on functional connectivity of the human brain. We focused on pre-determined anatomically distinct regions known to have a high density of opioid receptors and which are involved in opioid-induced central nervous (CNS) system effects. The primary aim of the study was to investigate how remifentanil affects the functional connectivity of the brain networks in the resting state fMRI and the temporal as well as spatial modes of these effects. Secondly, we wanted to analyse the effect of remifentanil-induced respiratory depression on connectivity and BOLD responses. Finally, we tested, by using three remifentanil boluses, whether the development of acute opioid tolerance can be seen in the BOLD responses.

Materials and methods

The study protocol was approved by the ethics committee of the Department of Surgery at the Helsinki University Central Hospital and by the Finnish Medicines Agency (now FIMEA).

Subjects

We recruited healthy right-handed subjects aged 18–45 years with no regular mediation or previous opioid misuse. Each subject gave a written informed consent for the participation in the study. Thereafter a psychological assessment was performed. This consisted of a structured interview including questions of medication use and allergies, and validated psychological questionnaires (Beck's depression inventory BDI-II, Pain Anxiety Symptoms Scale Pain Anxiety Symptoms Scale, (Beck *et al*, 1996; McCracken and Dhingra, 2002)). Exclusion criteria for participation in the study were a major psychiatric problem, substance abuse, a neurological disease or a chronic or recurrent pain condition. No medication was allowed on the day of the experiments, and the subjects fasted for at least two hours before the behavioural pre-test and the fMRI.

Of the total 20 of subjects who underwent the psychological assessment, one subject was excluded because of a concurrent pain condition and claustrophobic symptoms. Nineteen healthy subjects (8 males) proceeded to the behavioural pre-test in the pain clinic and the actual fMRI study. They were all right handed and 19–28 years (mean 24 years). Two subjects were later excluded from the analysis due to excessive head movements during fMRI image acquisition. Three were lost due to operator related technical errors arising during the imaging resulting in incomplete data. The remaining 14 subjects (6 males) were 19–28 years (mean 24 years). A behavioural pre-test was performed to monitor individually the physiological and possible adverse effects of remifentanil before the fMRI. No medication was allowed on the day of the experiments, and the subjects fasted for at least two hours before the behavioural pre-test and the fMRI.

Drug administration

An intravenous line was inserted into the antecubital vein of the left arm, and a slow infusion of Ringer's acetate was started. A remifentanil (Ultiva®, GlaxoSmithKline) dose of 0.3 μg/kg was used both in the behavioural pre-test and in the fMRI study. The drug was administered as a rapid intravenous bolus and flushed with 10 ml of

Ringer's acetate. Saline was used in the behavioural pre-test and the fMRI study to control possible nonspecific effects.

Behavioural pre-tests

As a safety precaution and to familiarize the subjects with the procedure behavioural pre-tests were performed in a post-anaesthesia care unit as in our earlier study (Leppä *et al*, 2006). Heart rate, blood pressure, blood oxygen saturation (SpO_2), and end-tidal carbon dioxide ($ETCO_2$) were monitored using a Datex-Ohmeda patient monitoring system (Helsinki, Finland). During the behavioural pre-test, three remifentanil and two saline boluses were administered intravenously in a single-blind fashion at 9-minute intervals (remifentanil, saline, remifentanil, saline, remifentanil). At 1-minute intervals, the subjects rated their subjective drug effect on a four-step scale (no effect, mild effect, mediocre effect, strong effect) and the readings of the SpO_2, $ETCO_2$, heart rate, and blood pressure were marked down. After the pre-tests, the subjects were asked about their subjective sensations and possible adverse effects during the test.

Imaging

The fMRI experiments were performed using a Siemens Avanto (Erlangen, Germany) 1.5 T scanner and a 12-channel head coil. The head was stabilized with a vacuum cushion to minimize movement artefacts. During the scanning, the subjects were instructed to avoid movement and to maintain visual fixation of their eyes on a fixation cross displayed on a rear-projection screen which was visible to the subjects through a mirror attached on the head coil. First, a structural image set was acquired using a T1-weighted three-dimensional MPRAGE sequence (176 sagittal 1 mm slice planes, field of view 224×256 mm, 224×256 matrix, TR 11 ms, TE 4.94 ms, flip angle 15). Then, fMRI measurements were performed using a gradient-echo echo-planar sequence with inter-leaved slice acquisition (48 axial slice planes with a thickness of 3 mm, no gap between slices, field of view 192 mm, 64×64 matrix, TE 40 ms, TR 3600 ms, flip angle 90). The axial slices of the functional image set extended from the superior edge of the brain below the basal parts of the cerebellum. The slices were parallel to a line from the base of the occipital lobe to the base of the frontal lobe in the mid-line sagittal localiser image. A time series of 760 functional image volumes was collected. The total imaging time was about 60 minutes.

During the fMRI experiments, three doses of remifentanil 0.3 μg/kg and two saline controls were administered in the same order as in the behavioural pre-tests (remifentanil, saline, remifentanil, saline, remifentanil). The subjects were unaware of the order of the injections (remifentanil or saline). Each dose was separated by 9 minute as in the behavioural pre-tests. Eight and a half minutes after each bolus injection the subjects were asked to rate the possible maximal drug effect on a four-step scale (no effect, mild effect, moderate effect, strong effect) that they experienced after the bolus injection. They were instructed to answer this question by moving their left hand fingers about their drug effect sensations. Heart rate, SpO_2 and $ETCO_2$ were continuously monitored using a Datex-Ohmeda patient monitoring system (Helsinki, Finland) suitable for MRI environment. The readings were marked down at 1-minute intervals. The averaged data from the three 9-minute periods in which remifentanil was administered were compared with the average of the two 9-minute saline periods.

Image pre-processing and registration

The anatomical reference scans were segmented with Advanced Normalization Tools software (http://www.picsl.upenn.edu/ANTS), which was used for the removal of non-brain tissues [Avants 2011].

Functional image pre-processing was performed with FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl)(Smith *et al*, 2004). Functional images were pre-processed by removing the first two image volumes to allow the longitudinal spin relaxation saturation. The functional image volumes were realigned with the MCFLIRT tool from FSL to correct for subject movement. Non-brain voxels were masked from the functional images with BET (Smith, 2002) from FSL.

The functional images were then spatially smoothed by a Gaussian smoothing kernel (5 mm full width at half maximum), intensity normalized by a single multiplicative factor and temporally filtered by Gaussian-weighted least squares straight line fitting (σ=1250 s).

The time series were then decomposed into independent components with MELODIC (Multivariate Exploratory Linear Decomposition into Independent Components) Version 3.10, part of FSL (Beckmann and Smith, 2004). Pre-processed data were whitened and projected into a multidimensional (62–168) subspace using probabilistic

principal component analysis where the number of dimensions was estimated using the Laplace approximation to the Bayesian evidence of the model order (Minka, 2000; Beckmann and Smith, 2004).

The components were evaluated by two investigators independently and later by consensus reading to identify components containing artefacts related to motion and scanner related artefacts and components that were confined to white matter, liquor spaces and venous sinuses. These components were removed from the data with *fsl_regfilt* program.

Before the group tensorial ICA analysis the data were split into time-series (five sessions) consisting of data acquired from the time of injection of the respective bolus to the start of the period of subjective rating of the response. The nine image volumes acquired during the subjective evaluation of the drug effect were discarded. Therefore, the image sets entering the ICA analysis contained 141 image volumes (507.6 s epochs). The ICA pre-processing also included demeaning of the data and normalization of the voxel-wise variance.

The pre-processed functional images were co-registered to individual structural image by using FLIRT tool from FSL. The structural images were co-registered to the standard MNI space by FLIRT. Registration from structural images to the standard MNI space was then refined using FNIRT non-linear registration tool from FSL (Andersson *et al*, 2007).

Group ICA analysis

A tensorial ICA analysis (Beckmann and Smith, 2005) was performed with MELODIC using epochs under all five conditions (three following the remifentanil boluses and two after the saline boluses). Higher-dimensional decomposition of all fMRI data sets into spatial, temporal and subject modes was performed with automatic model order selection and Gaussian/Gamma mixture-model-based inference on component maps. Subsequently the data were projected into an 8-dimensional subspace.

The session mode reflects the effect size within the session i.e. the magnitude of a component derived from tensorial ICA signal decomposition. A mixed effect ANOVA test was performed with the session mode domain values as fixed effect and subject as a random effect and t-tests were performed to test for differences between the effects of the three remifentanil injections and the two saline injections.

We performed temporal concatenation ICA analysis was performed by using the epochs after the saline bolus injections. Two epochs of each subject were temporally concatenated, followed by spatial ICA estimated by maximising non-Gaussian sources, using robust voxel-wise variance normalisation of data, automatic model order selection and Gaussian/Gamma mixture-model based inference on component maps. Eight dimensions were used in decomposition.

Estimation of subject specific temporal and spatial modes from the ICA maps from the temporal concatenation group level analysis was performed with template maps using spatial regression followed by temporal regression. This dual regression then resulted in 560 statistical parameter maps (14 subjects×5 boluses×8 resting state networks). These spatial maps were entered into a mixed effect generalized linear model. FSL *randomise* program was used to test differences between conditions with 5000 permutations. In a GLM model we tested differences between the saline boluses, the first, second and third remifentanil boluses with a paired t-test. Altogether 12 contrasts were evaluated in 8 components. To correct for these multiple comparisons we therefore used a Bonferroni corrected p-value of 0.01/12/8 for the volume-wise corrected p-value maps for delineation of areas with differences in connectivity.

Physiological measurements and drug effect ratings

Subjective ratings for remifentanil effects were evaluated in a mixed effect analysis of variance with bolus type as a fixed effect and subject as a random effect with R (version 3.3.2, www.r-project.org). As a measure of the effect of the drug bolus on the recorded physiological values we calculated the sum of values recorded from the time of the bolus injection to the last value before the next bolus. The sums of the $ETCO_2$, heart rate, and $Sp0_2$ values were fitted with a mixed effect linear model with the bolus type as a fixed effect and subject as random effect.

Results

Behavioural and physiological measurements

Subjective ratings for remifentanil effects differed significantly from the effects reported after saline boluses ($F=90.1$, $df=4$) but they were not significantly different between the three remifentanil boluses.

$ETCO_2$ varied between 6.4% and 4.5% peaking during the remifentanil boluses with a temporal behaviour closely corresponding to the predicted effect site concentration according to the PK/PD model by Minto (Minto *et al*, 1997). SpO_2 varied between 100% and 89% with the lowest values occurring during remifentanil boluses. Heart rate varied between 104 and 44 beats/min lowest values occurring during remifentanil boluses.

Tensor-ICA analysis

Similar areas as previously reported with hypercarbia.

Of eight different components three different types of temporal modes were observed. Temporal modes for components 1–4 fitted best the $ETCO_2$.

The hemodynamic response to BOLD signal decreased with repeated remifentanil bolus injections but only the difference between first and third as well second and third bolus in components 5 to 8 were significant with $p < 0.05$ when corrected for nine comparisons.

Resting state networks and dual regression analysis

The eight resting state networks detected from epochs after saline boluses included some commonly known brain networks found in existing literature.

The first remifentanil bolus compared with the saline boluses increased connectivity in all networks. After the second remifentanil bolus connectivity was greater within networks 4–8 compared with saline boluses. After the third remifentanil bolus, compared with saline boluses, larger connectivity was seen in networks 6–8. In network 4, however, the connectivity after the first and second remifentanil boluses was lower in the pre- and postcentral gyri compared with the saline boluses. Interestingly, also in network 8 in the pre- and postcentral gyri, precuneus and occipital cortex connectivity was lower after the second and third remifentanil boluses compared with the saline boluses.

After the first and second compared with the third remifentanil bolus connectivity was greater within network 3 in the anterior cingulate cortex, paracingulate, superior frontal and supramarginal gyri, parietal operculum and planum temporale.

Connectivity within network 3 was larger after the first remifentanil bolus compared with the third remifentanil bolus in the superior parietal lobule.

Conclusions

A gradual decrease in response to remifentanil bolus injection was observed, with the third repetition of bolus producing a significantly smaller hemodynamic response than the two first. This may reflect acute tolerance but needs further research.