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Key words (2-10) sensitivity, displacement, autoreceptor, 5-HT_{1B} receptors

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No correlation between serotonin and its metabolite 5-HIAA in the cerebrospinal fluid and [^{11}C]AZ10419369 binding measured with PET in healthy volunteers

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Brain microdialysis provides a mean to access extracellular monoamine concentrations in animals, although this method can rarely be applied in humans in vivo. Instead, displacement of radioligand binding during positron emission tomography (PET) has become an established way of detecting altered endogenous dopamine levels in response to pharmacological challenge(Laruelle, 2000). The application of PET to measure changes in serotonin levels has been hampered by a lack of suitable radioligands(Paterson et al., 2010). There are promising results with the recently developed 5-HT_{1B} receptor antagonist [^{11}C]AZ10419369, with decreased binding in response to pharmacologically induced serotonin release (Finnema et al., 2012) and high doses of a selective serotonin reuptake inhibitor (SSRI) in non-human primates(Nord et al., 2013). However, the effect of baseline serotonin levels on [^{11}C]AZ10419369 binding in humans has not yet been studied. This complicates the interpretation of clinical PET studies, as differences in BP_{ND} between groups may be explained by both differences in receptor density and endogenous ligand concentration.

Despite the advent of PET in the 1980s there are few studies addressing the relationship between imaging of the serotonin system in the brain and measurements of serotonergic activity in the cerebrospinal fluid (CSF). Samples from the CSF are obtained through lumbar puncture (LP). Due to technical limitations in the measurement of serotonin in the CSF(Anderson et al., 1990), its metabolite 5-hydroxyindoleacetic acid (5-HIAA) has been more frequently employed in studies of serotonergic activity, especially in relation to psychiatric disorders(Asberg, 1997). However, with improved quantification methods CSF serotonin has been resurrected as an estimation of extracellular serotonin

in the brain(Anderson et al., 2002; Hubbard et al., 2010).

The aim of this study was to test the sensitivity of [^{11}C]AZ10419369 to baseline endogenous serotonin levels, as estimated by concentrations of serotonin and its metabolite 5-HIAA in the CSF. Based on the literature we hypothesized a correlation between [^{11}C]AZ10419369 BP_{ND} and CSF serotonin, and secondly, also a correlation between 5-HIAA in the cerebrospinal fluid and [^{11}C]AZ10419369 binding.

The study was approved by the Regional Ethical Review Board in Stockholm and by the Radiation safety committee of the Karolinska University Hospital. Twelve healthy subjects (4 males, 8 females, median age 25 years, range 20-53) without psychiatric history were included after giving written informed consent. The subjects were healthy, according to medical history, physical examination, blood analysis, magnetic resonance imaging of the brain and structured psychiatrical assessment with M.I.N.I.(Sheehan et al., 1998)(M.T.).

Urine drug screenings for metamphetamine, cocaine, cannabis, bensodiazepines, methadone, barbiturates, amphetamine, opiates, phencyclidine and buprenorphine were performed before the PET examinations, to exclude the use of drugs at time of PET. The examination with PET and [^{11}C]AZ10419369 was performed as previously described(Varnas et al., 2011). The mean (± s.d.) injected dose of [^{11}C]AZ10419369 was 370.1 (± 47.3) MBq. The specific radioactivity of the radioligand injected varied between 86 and 327 GBq/mmol, corresponding to an injected mass between 0.52 and 2.22 µg. The PET examinations were performed using the High Resolution Research Tomograph, with the ordinary Poisson 3D ordered subset expectation maximization algorithm, with 10 iterations and 16 subsets(Varrone et al., 2009). List mode data were reconstructed as earlier described(Nord et al., 2013).

The magnetic resonance imaging (MRI) examinations were performed with a 3 Tesla system. The MRI protocol included a T2-weighted sequence to rule out pathology and a T1-weighted 3-dimensional sequence for optimal visualization of anatomy and coregistration with PET images.

In general, the head movements during the PET examination were minor and could be corrected for

with a frame-to-frame-realignment algorithm as previously described (Schain et al., 2012). For three subjects with larger head movements during PET, the PET images were reconstructed using frame specific attenuation-data.

SPM5 (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, U.K.) was used to coregister T1-weighted MRI-images to PET-images and segment them into grey matter, white matter and cerebrospinal fluid. The binding potential of the whole brain (WB) was chosen as the primary PET parameter, defined as the average BP_{ND} in the grey and white matter. In addition, regions of interest (ROIs) were also defined by the Automated Anatomical Labeling (AAL) template(Tzourio-Mazoyer et al., 2002) for the occipital cortex (OC) and the caudate nucleus(CN). The cerebellum was chosen as reference region, due to its negligible 5-HT_{1B} receptor density (Varnas et al., 2001), and defined manually. The simplified reference tissue model has been validated for calculation of [^{11}C]AZ10419369 BP_{ND} in humans(Varnas et al., 2011) and was applied within Matlab R2007b for Windows.

CSF samples were collected in the morning by lumbar puncture (P.S.) between L3/L4 with the patient in sitting position, within one month after PET. 5 ml of CSF was collected and immediately centrifuged at 2000 *g* for 10 min at room temperature, aliqouted in 1 ml aliquots and frozen at -80°C. All CSF samples were free from contamination with blood as determined with erythrocyte counts. The samples were then prepared for analysis with high performance liquid chromatography (HPLC, with Coulochem III electrochemical detector with a 5011A coulometric analytical cell (Dionex Ultimate 3000 series, ThermoFisher Scientific, Stockholm, Sweden) as previously described(Hubbard et al., 2010; Yang and Beal, 2011). The limit of detection was 0.16 ng/mL for serotonin and 0.23 ng/mL for 5-HIAA. Test-retest measurements for one representative analyte (5-HIAA) indicated a coefficient of variation of less than 1 % with this method.

To assess the relationship between global and regional BP_{ND}-values from the PET measurements, and 5-HIAA and serotonin in the CSF, Pearson's correlation was applied, in IBM SPSS Statistics 22 for Windows (SPSS Inc, Somers, NY, USA).

The mean serotonin concentration in the CSF (±s.d.) was 0,58 (±0.12) ng/ml. In one subject 5-HIAA levels in the CSF were below the limit of detection. The average 5-HIAA concentration (±s.d.) for the rest of the group was 1.49 (±1.22) ng/ml. For the whole comparable group (n=11) there was no significant correlation between 5-HIAA and serotonin concentrations (rho=0.448, p=0.167). When the two subjects with high 5-HIAA (>3 ng/ml) were removed from the analysis, 5-HIAA and serotonin clearly correlated (rho=0.938, p<0.001).

There was no significant correlation between CSF serotonin and binding potential in the whole brain (rho=0.231, p=0.471, figure A), in the caudate nucleus (rho=0.170, p=0.598) or in the occipital cortex (rho=0.185, p=0.565). 5-HIAA in the CSF did not correlate with BP_{ND} in WB (rho=0.143, p=0.675, figure B), in CN (rho=0.105, p=0.759) or in OC (rho=0.189, p=0.578).

There are to our knowledge no previously published studies relating serotonin levels in the cerebrospinal fluid to PET data. We found no significant correlations, between [^{11}C]AZ10419369 BP_{ND} in the whole brain or in the brain regions considered most relevant and serotonin or 5-HIAA in the CSF.

How sensitive is [^{11}C]AZ10419369 to baseline serotonin concentrations? The ability of [^{11}C]AZ10419369 and other serotonin receptor antagonists to measure physiological serotonin fluctuations has been questioned on a theoretical basis, since these radioligands, in contrast with the endogenous ligand serotonin, do not differentiate between the affinity states of the targeted receptors(Zimmer and Le Bars, 2013). Displacement of [^{11}C]AZ10419369 binding has been convincingly shown in non-human primates after pharmacological challenge with fenfluramine or high doses of an SSRI, which based on microdialysis studies on rodents are expected to yield more than twofold increases in serotonin concentrations(Rothman and Baumann, 2002). However, [^{11}C]AZ10419369 binding was not significantly reduced with a single, clinically relevant, dose of SSRI in healthy volunteers(Nord et al., 2013).

How well do levels of serotonin and 5-HIAA in the CSF correspond to extracellular serotonin in the brain? In animal studies, a correlation between serotonin in the CSF and in the brain has been

found (Matsumoto et al., 1991). Likewise, serotonin in the CSF correlated with brain levels in a human post-mortem material(Wester et al., 1990). In non-human primates a twofold increase in CSF serotonin was observed after administration of a selective serotonin reuptake inhibitor, providing indirect support for a correspondence between serotonin concentrations in extracellular fluid in the brain and in CSF(Anderson et al., 2002). For 5-HIAA, intraindividual correlations between the concentration in the CSF and in different brain regions have been demonstrated post mortem(Stanley et al., 1985; Wester et al., 1990). Stanley and coworkers thoroughly analyzed the lumbar puncture CSF 5-HIAA from their post-mortem material and found similar properties as in the range of antemortem studies(Stanley et al., 1985). Based on these data CSF levels of serotonin, and possibly its metabolite 5-HIAA, would be expected to correlate with brain serotonin concentrations. However, since [^{11}C]AZ10419369 binds selectively to 5-HT_{1B} receptors, endogenous displacement of radioligand binding would largely depend on serotonin concentrations in the synapses(Zimmer and Le Bars, 2013). It is difficult to assess to which degree average brain serotonin levels correspond to synaptic serotonin.

The median time elapsed between PET and LP was 20 days. The time limit 1 month between PET and LP was chosen for practical reasons. There are to our knowledge no test-retest studies on serotonin concentrations in human CSF. A pilot study in non-human primates claimed good longitudinal stability of CSF serotonin over a three month period, with variances of 16 and 20 % in the two monkeys exposed to repeated measurements(Anderson et al., 2002). Serial LPs have demonstrated good reproducibility of CSF 5-HIAA concentrations for a time period of 5 days(Ben Menachem et al., 1989). The distinct correlation between the concentrations of serotonin and 5-HIAA in the CSF, with the exception of the two outliers with high 5-HIAA levels, provides indirect support for the reproducibility of CSF serotonin measurements also in healthy human subjects.

In conclusion, we found no correlation between serotonin and its metabolite 5-HIAA in the cerebrospinal fluid and [^{11}C]AZ10419369 binding in this pilot PET study. Given sufficient longitudinal stability of concentrations of serotonin and 5-HIAA in the CSF, the results of the present

study do not support that the [11C]AZ10419369 binding potential reflects physiological serotonin and 5-HIAA levels in CSF. The serotonin detection threshold for [11C]AZ10419369 remains to be determined.

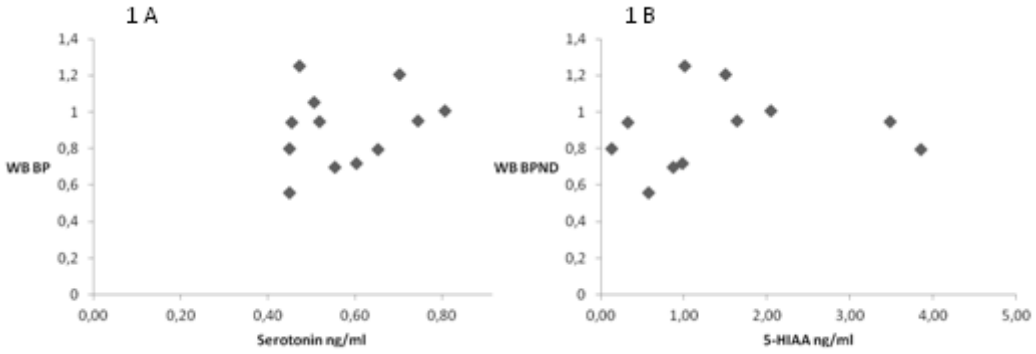

Figure legend. Scatterplots depicting the relationship between [11C]AZ10419369 BP_{ND} in the whole brain and serotonin(A) and 5-HIAA in the CSF (B).

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