

# Serotonin receptor binding in mild cognitive impairment studied by positron emission tomography and [<sup>18</sup>F]-altanserin

Steen G. Hasselbalch, Karine Madsen, Claus Svarer, Lars Pinborg, Søren Holm, Olaf B. Paulson, Gitte M. Knudsen & Gunhild Waldemar  
Memory Disorders Research Group & Neurobiology Research Unit, Department of Neurology, Rigshospitalet, Copenhagen University Hospital, Denmark



## ■ BACKGROUND

Evidence from postmortem human brain studies, clinical pharmacology, animals studies, and recently PET-studies in humans implicate dysfunction of the serotonergic transmitter system in AD (1), and this dysfunction seems to correlate to neuropsychiatric symptoms (2). One of the most consistent post mortem findings is a reduction in the number of the 5-HT<sub>2A</sub> receptor subtype (2). In vivo functional imaging studies have confirmed large reductions in 5-HT<sub>2A</sub> receptor binding in mild to moderately demented AD patients using positron emission tomography (PET) (3,4) or Single Photon Emission Tomography (SPECT) (5). The G-protein coupled 5-HT<sub>2A</sub> receptor is highly expressed in neocortex and less so in limbic cortex and basal ganglia. 5-HT<sub>2A</sub> receptors are associated with cholinergic and glutamatergic neurons in the cerebral cortex and hippocampus and seem to be implicated in cognition by regulation of the release of these and other neurotransmitters. In experimental studies, 5-HT<sub>2A</sub> agonists enhance and antagonists impair learning (6). Thus, dysfunction of the 5-HT<sub>2A</sub> receptor in AD may not only lead to neuropsychiatric disturbances, but also to cognitive dysfunction.

## ■ OBJECTIVE

The objective of this study was to assess if 5-HT<sub>2A</sub> receptor binding was decreased in amnesic MCI, and whether neuropsychiatric symptoms correlate with reduced 5-HT<sub>2A</sub> binding in specific regions. We deliberately chose a subtype of MCI that would reflect prodromal AD in order to exclude 5-HT<sub>2A</sub> receptor changes due to affective disease or other neuropsychiatric disorders. We hypothesized that serotonergic dysfunction sets in early in the course of the disease and is related to part of the symptomatology of very early AD.

## ■ METHODS

- SUBJECTS:** Sixteen amnesic MCI patients were recruited from the Copenhagen University Hospital Memory Clinic, which is an out-patient clinic based in neurology. MCI was defined using the criteria of Petersen et al. (7). Based on a neuropsychological test battery, episodic memory was the only cognitive domain with significant impairment in this group. Further, all MCI patients had normal basic functions on activities of daily living and a score on the Clinical Dementia Rating (CDR) of 0.5. Seventeen age-matched healthy control subjects were recruited from newspaper advertisements.
- PET:** PET studies were performed using a GE Advance camera in 3D mode, and 200-350 MBq [<sup>18</sup>F]-altanserin was administered using the bolus/infusion approach described by Pinborg et al. (8). Plasma input was corrected for metabolites by HPLC. The binding potential BP1 was defined as  $(C_{\text{ROI}} - C_{\text{cerebellum}}) / C_{\text{plasma}}$ , where  $C_{\text{ROI}}$  and  $C_{\text{cerebellum}}$  are steady-state mean count densities in the volume of interest and in the reference region, respectively, and  $C_{\text{plasma}}$  is the steady-state activity of non-metabolized tracer. BP1 was determined as the mean of 5 steady state measurements from 120-160 min after start of infusion.
- MRI:** MRI studies were performed using a Siemens 1.5 T<sub>1</sub> and T1 weighted images were obtained for coregistration and segmentation. Volumes of interest (VOI) Analysis was performed by an automated VOI method using MRI defined volumes (9). A partial volume correction was applied to segmented grey matter activity obtained by PET (10).
- CORRELATIONS:** Volumes of Interests (VOI's) were compared group-wise using Student's t-test and since a monotonous effect was found for most brain regions, correction for multiple statistical tests was not performed. Based on *a priori* hypotheses, we correlated cognitive and neuropsychiatric test with BP1 in specific regions. All correlations were performed with adjustment for age in a multiple linear regression analysis with the test score as the dependent variable and regional BP1 and age as independent variable. Since the correlation analysis tested only the specific hypotheses, p values were not adjusted for multiple statistical tests, except for MMSE and NPI where several VOI's were considered. A p-value < 0.05 was considered significant.

## ■ RESULTS

Subject demographics are shown in Table 1. 5-HT<sub>2A</sub> binding measured by PET is shown in Table 2.

TABLE 1. Demographics	Control subjects (n=17)	MCI subjects (n=16)
Age (range)	70 ±5 62-79	73 ±6 59-82
Gender (female/male)	8/9	7/9
MMSE score (range)	29.4 ±0.6 28-30	26.1 ±2.2* (23-30)
WMS Logical Memory Test score	50 ±10	14 ±11*
Verbal Fluency Test (number of words)	15 ±4	10 ±4*
Trail Making B Test (time to complete in sec)	107 ±75	134 ±78
NPI total score (range)	-	5.6 ±5.3 (0-16)
1 year clinical status (progressed/stable)	-	6/10

p < 0.05

TABLE 2. 5-HT <sub>2A</sub> binding measured with PET	Control subjects (n=17)	MCI subjects (n=16)	Difference
Orbitofrontal Cortex	1.91 ±0.55	1.42 ±0.65	-26%*
Medial/Inferior Frontal Gyri	2.76 ±0.63	2.15 ±0.68	-22%*
Superior Frontal Gyri	2.60 ±0.60	1.92 ±0.65	-26%*
Medial/Inferior Temporal Gyri	2.15 ±0.53	1.66 ±0.53	-23%*
Superior Temporal Gyri	2.38 ±0.62	1.85 ±0.57	-22%*
Parietal Cortex	3.08 ±0.73	2.28 ±0.68	-26%*
Occipital Cortex	2.80 ±0.82	2.17 ±0.67	-22%*
Primary Sensori-Motor Cortex	2.79 ±0.72	1.44 ±0.55	-29%*
Caudate nucleus	0.53 ±0.32	0.71 ±0.31	ns
Putamen	0.68 ±0.32	0.74 ±0.26	ns
Thalamus	0.55 ±0.33	0.61 ±0.45	ns
Insula	1.57 ±0.42	1.18 ±0.41	-25%*
Anterior Cingulate Gyri	1.87 ±0.47	1.36 ±0.54	-28%*
Posterior Cingulate Gyri	2.08 ±0.61	1.49 ±0.56	-28%*
Entorhinal Cortex	0.56 ±0.31	0.44 ±0.33	ns
Hippocampus	0.26 ±0.24	0.37 ±0.21	ns

Values shown in Table 2 are mean ±1SD of grey matter voxels in VOIs. Values were corrected for partial volume effect (10). Because of no left-right differences, average of left and right VOIs are shown. \*: p < 0.05, Student's T-test without correction for multiple statistical tests. ns: not significant

In most of the VOI's studied, 5-HT<sub>2A</sub> receptor binding was significantly reduced in patients with MCI. In neocortex, the mean reduction in BP1 was 31% without, and 25% with correction for partial volume. Thus, only a minor fraction of the reduction in BP1 could be explained by the presence of atrophy. For the subcortical brain structures, no significant reductions in BP1 could be identified. Striatal 5-HT<sub>2A</sub> binding correlated positively with MMSE score, as an indication that a poorer global cognitive function is related to a reduced striatal 5-HT<sub>2A</sub> binding (Table 3). However, when corrected for multiple statistical testing, this correlation was no longer significant. In prefrontal cortex, there was a trend for a correlation between a poorer performance on Trail Making B Test and a reduced 5-HT<sub>2A</sub> binding. Depression and anxiety scores from the Neuropsychiatric Inventory correlated with reduced 5-HT<sub>2A</sub> binding in prefrontal cortex and striatum, and the correlation with striatum remained significant after correction for multiple statistical tests (Figure 1 and Table 3).

Table 3. Correlations between 5-HT <sub>2A</sub> binding and test scores	Orbito-frontal	Prefrontal	Mesial Temporal	Anterior Cingulate	Striatum
MMSE	0.194	0.349	0.110	0.259	0.526*
WMS Logical memory	-	-	-0.178	-	-
Verbal Fluency	-	0.390	-	-	-
Trail B	-	-0.504	-	-	-
NPI depression+anxiety	-0.376	-0.523*	-	-0.434	-0.649**

Table 3: Correlations between VOIs (grouped in larger functionally related regions) and cognitive/psychiatric symptoms. R = partial correlations from multiple linear regression analysis with the test score as the dependent variable and regional 5-HT<sub>2A</sub> receptor binding potential, and age as covariates, \*: p < 0.05, \*\*: p < 0.05 corrected for multiple statistical tests.

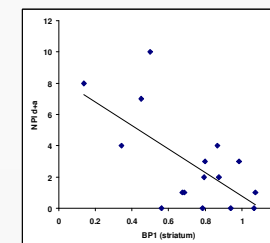


Figure 1: Correlation between 5-HT<sub>2A</sub> binding in striatum and depression/anxiety subscores of NPI (Neuropsychiatric Inventory). X-axis: BP1, binding potential of 5-HT<sub>2A</sub> receptors in grey matter in striatum (putamen and caudate nuclei). Y-axis: combined score of depression and anxiety from NPI (high scores= more severe and/or frequent symptoms)

## ■ DISCUSSION

Whereas a significant and uniform reduction was found in the 5-HT<sub>2A</sub> binding in large neocortical regions, no consistent findings were obtained from smaller regions, probably due to higher variability in BP1 in small regions with lower binding. The reduction in 5-HT<sub>2A</sub> binding was widespread and comparable to that found in more advanced stages of AD (4-6). Albeit weak, our data suggests an association between reduction in 5-HT<sub>2A</sub> receptor binding and poorer cognitive function. The stronger correlation between dysphoria/anxiety symptoms and 5-HT<sub>2A</sub> binding in striatum imply that an alteration of the serotonergic modulation of fronto-striatal circuits may be implicated in the neuropsychiatric symptoms of very early AD.

In the group of MCI patients, other neuropsychiatric symptoms, such as aggression, irritability, and apathy were infrequent symptoms, and the relationship between these symptoms and 5-HT<sub>2A</sub> binding remains to be elucidated.

## ■ ACKNOWLEDGEMENTS

The authors thank the Danish 1991 Pharmacy Foundation and the Danish Health Insurance Fund for financial support to the research programs in the Memory Disorders Research Unit. The study was supported by The 1991 Pharmacy Foundation, The Health Insurance Foundation, The Lundbeck Foundation, Danish Medical Research Council. The John and Birthe Meyer Foundation is gratefully acknowledged for the donation of the Cyclotron and PET-scanner. The study was funded in part by the EC - FP5-project MCI-MCI, QLK6-CT- 2000-00502.

## ■ REFERENCES

- Meltzer CC, Smith G, DeKosky ST, et al. Serotonin in aging, late-life depression, and Alzheimer's disease: the emerging role of functional imaging. *Neuropsychopharmacology* 1998;18:407-430.
- Hasselbalch S, Knudsen GM. Imaging of neurotransmitter systems in dementia. In: The Dementias: Early Diagnosis and Evaluation (Herholz K, Perani D, Morris CM, eds) Blackwell Publishers, 2006.
- Blin J, Baron JC, Dubois B, et al. Loss of brain 5-HT<sub>2</sub> receptors in Alzheimer's disease. In vivo assessment with positron emission tomography and [<sup>18</sup>F]setoperone. *Brain* 1993;116 ( Pt 3):497-510.
- Meltzer CC, Price JC, Mathis CA, et al. PET imaging of serotonin type 2A receptors in late-life neuropsychiatric disorders. *Am J Psychiatry* 1999;156:1871-1876.
- Versijpt J, Van Laere KJ, Dumont F, et al. Imaging of the 5-HT<sub>2A</sub> system: age-, gender-, and Alzheimer's disease-related findings. *Neurobiol Aging* 2003;24:553-61.
- Williams GY, Rao SG, Goldman-Rakic PS. The physiological role of 5-HT<sub>2A</sub> receptors in working memory. *J Neurosci* 2002;22:2843-54.
- Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome [published erratum appears in *Arch Neurol* 1999 Jun;56(6):750]. *Arch Neurol* 1999;56:303-308.
- Pinborg LH, Adams KH, Svarer C, et al. Quantification of 5-HT<sub>2A</sub> receptors in the human brain using [<sup>18</sup>F]altanserin-PET and the bolus/infusion approach. *J Cereb Blood Flow Metab* 2003;23:985-96.
- Svarer C, Madsen K, Hasselbalch SG, et al. MR-based automatic delineation of volumes of interest in human brain PET images using probability maps. *Neuroimage* 2005;24:1969-79.
- Muller-Gartner HW, Link JM, Prince JL, et al. Measurement of radiotracer concentration in brain grey matter using positron emission tomography: MRI-based correction for partial volume effects. *J Cereb Blood Flow Metab* 1992;12:571-83.

