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NeuroImage

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NeuroImage 30 (2006) 341 – 348

Increased serotonin receptor availability in human sleep: Evidence from an [^{18}F]MPPF PET study in narcolepsy

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Received 24 May 2005; revised 19 September 2005; accepted 23 September 2005
Available online 4 November 2005

Data from animal studies suggest that serotonin release promotes wakefulness and suppresses REM sleep, but there are dangers in extrapolating these findings to humans. Binding of the radioligand [^{18}F]MPPF to 5HT$_{1A}$ receptors is sensitive to levels of endogenous serotonin. In this study, we aimed to demonstrate changes in serotonin receptor availability in the human brain in wakefulness and sleep using [^{18}F]MPPF and positron emission tomography. 14 subjects with narcolepsy cataplexy underwent [^{18}F]MPPF PET scans in wakefulness and in sleep. Subjects who used the stimulant methylphenidate took their normal medication for the wake scan but omitted it prior to the sleep scan. The change in binding potential (BP) between the sleep and wake scans was examined using paired *t* test. Methylphenidate is thought to have little or no effect on serotonergic neurotransmission, and in order to confirm the absence of an effect on [^{18}F]MPPF binding, a concurrent study was performed using a β-microprobe technique to examine the effect of methylphenidate administration on [^{18}F]MPPF binding in Sprague–Dawley rats. The human study showed a significant increase in [^{18}F]MPPF binding in sleep compared to wakefulness in the whole brain and all regions of interest examined (temporal cortex, mesial temporal region and cingulate cortex). The β-microprobe study confirmed that methylphenidate administration had no effect on [^{18}F]MPPF binding. These findings indicate that serotonin receptor availability is increased in sleep compared to wakefulness in narcoleptic humans.

Keywords: Serotonin; Sleep; PET; MPPF; Receptor; Binding potential

Serotonin, interacting with other neurotransmitters such as acetylcholine and noradrenalin, appears to promote wakefulness and suppress REM sleep. The serotonergic system is phylogenetically ancient and is found in all mammalian species. The majority of serotonergic cell bodies are located in the raphe nuclei, which lie around the midline of the brainstem, but these have widespread projections throughout the brain. Previous animal studies have suggested that the system is a fundamental component in the neurochemical regulation of sleep. Activity demonstrated with single unit recordings from serotonergic neurons in the dorsal raphe nucleus (McGinty and Harper, 1976; Trulson and Jacobs, 1979; Cespuglio et al., 1981) is tightly linked to the stage of sleep. The neurons are active in wakefulness, becoming progressively less active during non-REM sleep, and are essentially quiescent during REM sleep. Microdialysis studies in cats and rats (Portas and McCarley, 1994; Portas et al., 1998; Park et al., 1999; Strecker et al., 1999) have demonstrated changes in serotonin concentration which parallel the changes in neuronal firing rates.

Most available information about changes in serotonergic neurotransmission through the sleep–wake cycle has been obtained in experimental animals. There are dangers in extrapolating this to humans given our existing knowledge of interspecies differences. For example, serotonin exerts dramatic modulatory effects on circadian rhythms in hamsters but not mice (Antle et al., 2003), and lesions of the dorsal raphe nucleus have been shown to reduce sleep in cats (Jouvet, 1972) but not in rats (Bouhuys and Van Den Hoofdakker, 1977). The only human data on serotonin release during the sleep–wake cycle to our knowledge are in a single case report showing that the serotonin concentration in the CSF from the lateral ventricles was greatest in wakefulness and lowest in REM sleep (Zeitzer et al., 2002).

In light of the paucity of human data, we aimed to evaluate endogenous serotonin release in wakefulness and sleep in the

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Available online on ScienceDirect (www.sciencedirect.com).

1053-8119/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuroimage.2005.09.052

living human brain using the $5HT_{1A}$ PET ligand [^{18}F]MPPF. We hypothesized that [^{18}F]MPPF binding would be greater in sleep than in wakefulness, reflecting a reduction in endogenous serotonin release during sleep.

Materials and methods

Part I: effect of sleep on [^{18}F]MPPF binding: human study

Subjects

One of the problems faced by functional neuroimaging studies of human sleep is the lack of predictability with which spontaneous sleep occurs. We chose to study patients with narcolepsy as a way of circumventing this problem. Fourteen subjects with a diagnosis of narcolepsy (12 females, 2 males; mean age ± standard deviation: 52 years ± 14; age range: 26–67 years) were studied. The clinical features of the subjects are described in Table 1. The following inclusion criteria were applied: age over 18 years; diagnosis of narcolepsy made by a consultant neurologist following appropriate investigation; treatment with either methylphenidate alone or no medication. Subjects were excluded from the study if they had taken neuroleptic, antidepressant or stimulant medication other than methylphenidate within 1 month of enrolment; if there was a history of other neurological or psychiatric illness; or if the subject was pregnant. Subjects underwent a medical interview prior to enrollment in the study. In view of the potential effects of mood and serum tryptophan on endogenous serotonin release, all subjects completed the Beck Depression Inventory (BDI) and the Beck Anxiety Inventory (BAI) on the day of each PET scan. They were supplied with standardized meals for 24 h prior to each scan (no caffeinated drinks were permitted during this period), and serum tryptophan levels were measured 30 min prior to each scan. All subjects gave their written informed consent to the study protocol, which was approved by the medical ethics committee of the Austin Hospital.

Radiochemistry

[^{18}F]MPPF was obtained by nucleophilic substitution of the aromatic nitro group using previously described methods (Le Bars et al., 1998). The purity of [^{18}F]MPPF was greater than 99% on each synthesis, and specific activity ranged from 873 to 3300 mCi/μmol.

PET

Each patient underwent two [^{18}F]MPPF scans (total injected dose 4 mCi), one while taking their usual doses of methylphenidate (during which the subject was required to stay awake) and one after the subject had omitted methylphenidate on the day of the scan (during which the subject was allowed to fall asleep in the scanner prior to tracer injection). Each scan was carried out with polysomnographic monitoring (EEG, EOG and EMG) to monitor sleep state during the acquisition period. The PET scans were performed between 2 and 3 pm in every case, with the two scans being performed 1 week apart. Dynamic PET scans comprising 26 frames were acquired over 60 min using an ECAT positron tomograph (951/31R, CTI Siemens, Knoxville, TN, USA). The scanner was operated in 3D mode. Head movement was minimized using a molded head rest and head restraint. The data were reconstructed using the Kinahan and Rogers 3D reprojection algorithm (Kinahan and Rogers, 1989) with a Hanning filter. Attenuation correction was performed using data from a 10-min transmission scan with gallium-68/germanium-68 sources. The final image comprised 128 × 128 × 31 slices where the pixel size was 2.34 mm and the slice thickness was 3.37 mm.

MRI

MRI acquisition consisted of a high resolution three-dimensional T1-weighted image comprising 120 contiguous slices of 1 mm thickness and 1 mm × 1 mm pixel dimensions, with the anatomical volume covering the whole brain. MRI scans were acquired on a 1.5 T Signa Echospeed Superconducting Imaging System (General Electric Medical Systems, Milwaukee, WI). The three-dimensional spoiled gradient recalled echo acquisition comprised TR 10.5 ms, TE 2.2 ms, TI 350 ms, flip angle 20°, FOV 25 cm, matrix 256 × 256, NEX 1.

Data analysis

Kinetic analysis. A parametric image, obtained by estimating the binding potential (BP) and K_1 ratio (R_0) for each voxel, was

Table 1
Summary of clinical and treatment information for subjects enrolled in the study, with details of sleep duration in 'wake' and 'sleep' PET scans

Sex	Age (years)	Methylphenidate dose (mg/day)	%Age of time spent in sleep		Classification	Mean [^{18}F]MPPF BP (Whole Brain)	
			Wake scan	Sleep scan		Wake scan	Sleep scan
M	51	30	0	84	Good sleeper	0.223	0.241
F	54	45	3	93	Good sleeper	0.196	0.240
F	62	30	0	87	Good sleeper	0.188	0.212
F	59	90	0	85	Good sleeper	0.237	0.283
F	27	40	0	93	Good sleeper	0.318	0.349
F	26	Nil	0	84	Good sleeper	0.238	0.278
F	35	Nil	0	89	Good sleeper	0.272	0.276
F	66	30	0	19	Poor sleeper	0.228	0.234
F	56	50	0	34	Poor sleeper	0.212	0.199
F	29	50	0	21	Poor sleeper	0.191	0.194
M	52	10	8	26	Poor sleeper	0.235	0.264
M	66	70	0	29	Poor sleeper	0.265	0.265
F	58	70	0	52	Poor sleeper	0.224	0.212
F	67	40	0	30	Poor sleeper	0.284	0.281

Mean [^{18}F]MPPF BP for the whole brain is given for each subject. Note that all subjects have a diagnosis of narcolepsy/cataplexy.