Sex-related differences in the muscarinic acetylcholinergic receptor in the healthy human brain
—A positron emission tomography study—

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We evaluated the sex-related differences in the decline of the cerebral muscarinic acetylcholinergic receptor (mACH-R) due to aging by using $^{11}$C-N-methyl-4-piperidyl benzilate ($^{11}$C-NMPB) and positron emission tomography (PET). The subjects consisted of 37 (20 males and 17 females) healthy volunteers. The $^{11}$C-NMPB uptake was evaluated by the ratio method (regional $^{11}$C-NMPB uptake/Cerebellar $^{11}$C-NMPB uptake; rNMPB ratio). The correlation between sex, aging, and the rNMPB ratio in normal aging was evaluated by a multiple regression analysis. The rNMPB ratio was higher in females than in males throughout the entire cerebral region ($p < 0.01$–$p < 0.0001$) and the rNMPB ratio might thus possibly decline with age more rapidly in females. Our study therefore revealed the existence of sex-related differences in the cerebral mACH-R.

Key words: cerebral muscarinic acetylcholinergic receptor, normal aging, sex-related differences, C-11-NMPB, positron emission tomography

INTRODUCTION

The “CHOLINERGIC HYPOTHESIS OF GERIATRIC MEMORY DYSFUNCTION” was first presented by Bartus in 1982.¹ Up to now there have been many reports on cerebral muscarinic acetylcholine receptor (mACH-R) in normal aging but, little is still known about the sex-related differences in mACH-R. $^{11}$C-N-methyl-4-piperidyl benzilate (NMPB) is a ligand which shows a greater brain uptake and higher affinity for the mACH-R in the brain.² This ligand was first synthesized in a positron emission tomography (PET) study by Mulholland et al.,³ and a high uptake into the human cortices and basal ganglia was confirmed by Koepp et al.⁴ In the present study, sex-related differences in the decline in mACH-R due to aging were evaluated by $^{11}$C-NMPB and PET.

SUBJECTS AND METHOD

The subjects consisted of 37 volunteers, 20 males and 17 females (aged 24 to 75 years), who were either social workers, medical doctors or paramedical staff members working at Kyushu University Hospital. The volunteers who were all over 50 years old were selected after undergoing intellectual tests and brain imaging such as CT and MRI. As a result, all met the criteria of normal according to the mini-mental state examination,⁵ the Hasegawa Dementia Rating Scale⁶ and the Wechsler Adult Intelligence Scale, revised. Any subjects who showed signs of cerebrovascular disease on computed tomography or magnetic resonance imaging were excluded.

$^{11}$C-NMPB was synthesized by $N$-methylation of 4-piperidyl benzilate with $^{11}$C-methyl iodide according to the method of Suhara et al.⁷ The radiochemical purities were 98% and the specific activities ranged from 0.11 to 4.61 GBq/μmol.

PET studies were performed with a PET system HEADTOME-III (Shimadzu Corp., Kyoto, Japan) with a spacial resolution of 8.2 mm full width at half maximum.
(FWHM) which can simultaneously obtain 5 contiguous slices. A transmission scan with a $^{68}$Ge/$^{68}$Ga ring source was obtained previous to the emission scans for the correction of attenuation. The $^{11}$C-NMPB PET data were obtained for 15 min (from 85 to 100 min) after the administration of 185 to 1277 MBq of $^{11}$C-NMPB at levels of +20 mm, +35 mm, +50 mm, +65 mm, and +80 mm above the orbitomeatal line (Fig. 1).

The regions of interest (ROIs) were established on the $^{11}$C-NMPB images (the cerebellum (14 × 18 mm), the frontal, the temporal, the parietal (14 × 18 mm) and the

**Table 1** Age, body weight, injected dose and specific activity between males and females

<table>
<thead>
<tr>
<th></th>
<th>age (mean ± SD)</th>
<th>body weight (kg) (mean ± SD)</th>
<th>dose (MBq) (mean ± SD)</th>
<th>specific activity (GBq/μmol) (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>males (n = 20)</td>
<td>44.8 ± 18.0</td>
<td>61.8 ± 10.5</td>
<td>19.3 ± 4.1</td>
<td>457 ± 258</td>
</tr>
<tr>
<td>females (n = 17)</td>
<td>53.5 ± 13.3</td>
<td>50.8 ± 7.9**</td>
<td>21.3 ± 4.3</td>
<td>393 ± 168</td>
</tr>
</tbody>
</table>

****: p < 0.01

**Fig. 1** Regions of interest (ROIs) in the $^{11}$C-NMPB uptake images in a 70-year-old male normal volunteer. 1, 2: cerebellum, 3, 4: temporal cortex, 5, 6: hippocampus, 7, 8: frontal cortex, 9, 10: striatum, 11, 12: occipital cortex, 13, 14: parietal cortex. OM: orbitomeatal line

**Fig. 2** We plotted the rNMPB ratio (vertical axis) according to sex (males: open circle; females: closed circle) and aging (horizontal axis) in the 6 cerebral regions. In the whole cerebrum, a significant negative correlation between age and the rNMPB ratio was seen (p < 0.001–p < 0.0001) and the rNMPB ratio was found to be higher in females than in males throughout the entire cerebral region (p < 0.01–p < 0.0001).
Table 2  Multiple regression analysis between sex, aging and rNMPB ratio in normal volunteers

<table>
<thead>
<tr>
<th>region</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortex</td>
<td>0.35**</td>
<td>-0.023****</td>
<td>3.9***</td>
<td>0.81****</td>
</tr>
<tr>
<td>Temporal cortex</td>
<td>0.44***</td>
<td>-0.020****</td>
<td>3.6***</td>
<td>0.83****</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>0.43***</td>
<td>-0.022****</td>
<td>3.9***</td>
<td>0.76****</td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>0.46***</td>
<td>-0.016****</td>
<td>3.7***</td>
<td>0.67****</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.34***</td>
<td>-0.017****</td>
<td>3.1***</td>
<td>0.78****</td>
</tr>
<tr>
<td>Striatum</td>
<td>0.41**</td>
<td>-0.024****</td>
<td>4.4***</td>
<td>0.75****</td>
</tr>
</tbody>
</table>

multiple regression equations: \( Y = aX_1 + bX_2 + c \)
\( Y: \) rNMPB ratio (\( n = 37, m = f = 20:17 \))
\( X_1: \) Sex (male = 0, female = 1), \( X_2: \) Age (years)
a, b: coefficients of slope, c: coefficient of intercept, r: coefficient of correlation
*: p < 0.05, **: p < 0.01, ***: p < 0.001, ****: p < 0.0001

occupial (10 × 18 mm) cortices, the hippocampus (14 × 14 mm) and the striatum (multangular shape)) (Fig. 1). The mean values in the ROIs on both sides were averaged into a single value.

We analyzed the cerebral \(^{11}\)C-NMPB uptake by the ratio method,\(^8\) or the ratio of radioactivity in a region of interest to that in the cerebellum (regional \(^{11}\)C-NMPB uptake ratio; rNMPB ratio) for 15 min (from 85 to 100 min) after injection as in our previous study.\(^9\)

The rNMPB ratio for sex-related differences in the decline in mACH-R due to aging was evaluated by a multiple regression analysis and the difference between males and females in the slope of equations in the rNMPB ratio according to aging in each cerebral region was evaluated by a one-way analysis of covariance (ANCOVA).\(^10\)

multiple regression equations
\[ Y = aX_1 + bX_2 + c \]
\( Y: \) rNMPB ratio
\( X_1: \) sex (male = 0, female = 1), \( X_2: \) age (years)
a, b: coefficients of the slope, c: coefficient of the intercept

The correlations in comparisons were assessed at a significance threshold of \( p < 0.05 \).

This study was approved by the committee for the clinical application of cyclotron-producing radionuclides in Kyushu University Hospital, and informed consent was obtained before the study.

RESULTS

1. The differences between the backgrounds for males and females

The differences between males and females in age, body weight, injected dose and specific activity are shown in Table 1. No significant difference was observed except for body weight (\( p < 0.01 \)).

2. The rNMPB ratio for sex-related differences in the decline in mACH-R due to aging

We plotted the rNMPB ratio according to sex and aging (Fig. 2) and calculated multiple regression equations in each cerebral region (Table 2). In the whole cerebrum, a significant negative correlation between age and the rNMPB ratio was seen (\( p < 0.001 \)–\( p < 0.0001 \)) and the rNMPB ratio was higher in females than in males throughout the entire cerebral region (\( p < 0.01 \)–\( p < 0.0001 \)). There was no significant difference between males and females in the slope of equations for the rNMPB ratio according to aging in each cerebral region.

DISCUSSION

1. A decline in mACH-R due to aging

Several studies with mACH-R tracers were performed to evaluate the effects of aging on human cerebral cholinergic receptors. Postmortem human studies with \(^3\)H-QNB revealed a decline in mACH-R binding with aging in the frontal cortices and striata.\(^11,12\) Human in vivo studies with PET also revealed a decline in mACH-R in normal aging. A decline in the receptor binding with aging throughout the cerebrum was seen in a human in vivo study with \(^{11}\)C-benztropin (Dewey et al.\(^13\)) and \(^{11}\)C-NMPB (Suhara et al.\(^1\)). Our findings support those results, but Lee et al. reported no substantial age-related changes in the cerebrum in a human in vivo study with \(^{11}\)C-tropanyl benzilate (TRB).\(^14\) This contrasting result might be due not only to differences in the mACH-R tracers, but also to differences in the subject groups. According to Lee’s paper, their subjects included both males (\( n = 9 \)) and females (\( n = 5 \)), but, on the other hand, Dewey’s (\( n = 7 \)) and Suhara’s (\( n = 18 \)) subjects included only males.\(^7,13,14\)

In our study, the rNMPB ratios were about 12% higher in females than in males. Such sex-related differences in the human mACH-R and the small number of subjects might thus have led to Lee’s findings demonstrating statistical insignificance. Actually, in the single regression analysis between the rNMPB ratio and aging, a significant negative correlation was seen to exist due to the large number of subjects in our series (\( n = 37 \)), but coefficients of correlation were decreased throughout the entire cerebral region.
2. Sex-related differences in mACH-R
Several neuroimaging studies have revealed sex-related differences in cerebral blood flow (CBF)\textsuperscript{15} and glucose metabolism.\textsuperscript{16–18} One explanation of these sex-related differences could be the influence of estrogens,\textsuperscript{19} whereas another explanation could be the larger brain size in males than females.\textsuperscript{20} Nevertheless, little is known about the sex-related differences in neurotransmission, especially in cerebral mACH-R. In our human study, the nNMBP ratio was higher in females than in males throughout the entire cerebral region. Changes in the affinity and number of mACH-R during the estrous cycle have been reported in the rat.\textsuperscript{21} Olsen et al. reported the ability of estrogen to induce mACH-R binding in the central nervous system.\textsuperscript{22} As a result, the loss of the ovarian function has a negative impact on basal forebrain cholinergic neurons.\textsuperscript{23} A recent study suggested that women are at greater risk of Alzheimer’s disease than men.\textsuperscript{24} In our study, the slope of equations in the nNMBP ratio due to aging was found to be steeper in females than in males, but the difference was not regarded as significant because of the small number of subjects. If a steeper slope of the decline in cerebral mACH-R due to aging actually exists in females, then the sex-related differences in cerebral mACH-R might partly explain why postmenopausal women thus appear to be at greater risk for Alzheimer’s disease.

3. Effect of cerebral blood flow
In neuroreceptor imaging, the CBF was seen to deliver ligand into brain tissue through the blood-brain barrier and thereby affect the ligand-receptor binding. The ligand kinetics should therefore be determined in vivo to accurately evaluate receptor binding. The ratio method is simple but easily biased based on the blood-brain barrier transport compared to the 3- or 4-compartment model analysis,\textsuperscript{25} and the ratio method is also biased due to the change in cerebellar blood flow because the cerebellum was used as the standard region. The CBF effect on the nNMBP ratio must thus be considered when we interpret the data obtained. Rodriguez et al. showed an 11% higher CBF level in women than in men.\textsuperscript{15} Our results (12% higher nNMBP level in the women) therefore appear to be consistent with the CBF results of Rodriguez et al., unless we take the sex-related differences in cerebellar blood flow into account (Rootwelt et al. reported 9% higher cerebellar blood flow in women\textsuperscript{26}). A priori study reported that the decline in regional CBF (rCBF) due to aging was about a 0.5%/per year and the decline in cerebellar blood flow was about a 0.2%/per year.\textsuperscript{27} As a result, the decline in the nNMBP ratio (0.6%/per year) was about twice that in the CBF effect (rCBF/cerebellar blood flow) and not only the change in the CBF but also the change in mACH-R was reflected in our results.

4. Effect of cerebral volume
When we measure the neurofunctional values with PET, we must consider the effect of the cerebral volume. The decrease in cerebral volume to aging measured by MRI was reported by Gur et al.\textsuperscript{28} Their result (approximately 0.2%/per year) was smaller than the decline in the nNMBP ratio to aging (0.6%/per year). On the other hand, Coffey et al. reported greater age-related decreases in the parieto-occipital volume for men than women.\textsuperscript{29} Their result was in contrast to our result indicating a steeper decline in the parietal and occipital nNMBP ratio due to aging in women than in men. Therefore our results may partly include the effect of cerebral atrophy, but the change in mACH-R was also reflected.

CONCLUSION
Our study therefore suggests that sex-related differences in the decline due to aging do exist in the human cerebral mACH-R and we must therefore take the sex ratio of subjects into consideration when clinically evaluating mACH-R.

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