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

Tsuyoshi YOSHIDA,* Yasuo KUWABARA,* Masayuki SASAKI,* Toshimitsu FUKUMURA,** Atsushi ICHIMIYA,*** Masashi TAKITA,*** Koji OGOMORI,*** Yuichi ICHIYA**** and Kouji MASUDA*

*Departments of *Clinical Radiology and ***Neuropsychiatry, Graduate School of Medical Sciences, Kyushu University*
***The Japan Steel Works, Ltd.*
*****Department of Radiology, National Kyushu Cancer Center*

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 Koeppe 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.

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For reprint contact: Tsuyoshi Yoshida, M.D., Department of Clinical Radiology, Graduate School of Medical Sciences, Kyushu University, 3–1–1 Maidashi, Higashi-ku, Fukuoka 812–8582, JAPAN.

## 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 Scale[6] 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-methyliodide 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/$\mu$mol.

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

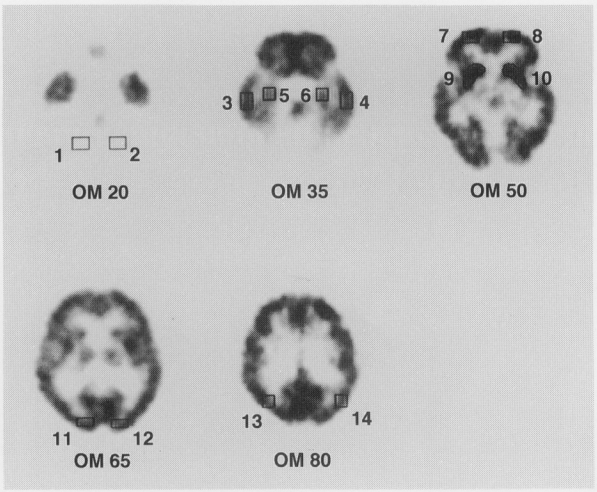


**Fig. 1** Regions of interest (ROIs) in the $^{11}C$-NMPB uptake images in a 70-year-old male normal volunteer. 1, 2: cerebellum, 3, 4: temporal cortex, 5, 6: hippocampus, 7, 8: frontal cortex, 9, 10: striatum, 11, 12: occipital cortex, 13, 14: parietal cortex. OM: orbitomeatal line

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

| sex | age (years) (mean ± SD) | body weight (kg) (mean ± SD) | dose (MBq) (mean ± SD) | specific activity (GBq/μmol) (mean ± SD) |
|---|---|---|---|---|
| males (n = 20) | 44.8 ± 18.0 | 61.8 ± 10.5 | 19.3 ± 4.1 | 457 ± 258 |
| females (n = 17) | 53.5 ± 13.3 | 50.8 ± 7.9** | 21.3 ± 4.3 | 393 ± 168 |

**: $p < 0.01$

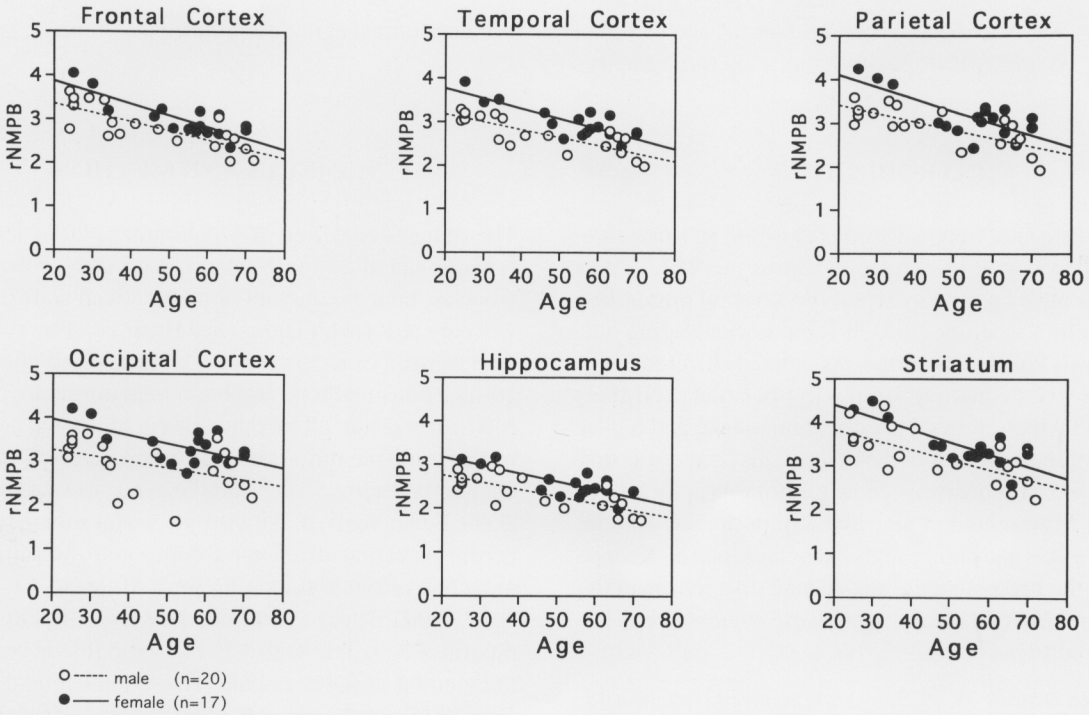


**Fig. 2** We plotted the rNMPB ratio (vertical axis) according to sex (males: open circle; females: closed circle) and aging (horizontal axis) in the 6 cerebral regions. In the whole cerebrum, a significant negative correlation between age and the rNMPB ratio was seen ($p < 0.001$–$p < 0.0001$) and 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

| region | *a* | *b* | *c* | r |
|---|---|---|---|---|
| Frontal cortex | 0.35*** | −0.023**** | 3.9**** | 0.81**** |
| Temporal cortex | 0.44**** | −0.020**** | 3.6**** | 0.83**** |
| Parietal cortex | 0.43*** | −0.022**** | 3.9**** | 0.76**** |
| Occipital cortex | 0.46*** | −0.016*** | 3.7**** | 0.67**** |
| Hippocampus | 0.34*** | −0.017**** | 3.1**** | 0.78**** |
| Striatum | 0.41** | −0.024**** | 4.4**** | 0.75**** |

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
*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$

occipital (10 × 18 mm) cortices, the hippocampus (14 × 14 mm) and the striatum (multiangular 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.[7]). 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 coefficinents 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)[15] and glucose metabolism.[16–18] One explanation of these sex-related differences could be the influence of estrogens,[19] whereas another explanation could be the larger brain size in males than females.[20] Nevertheless, little is known about the sex-related differences in neurotransmission, especially in cerebral mACh-R. In our human study, the rNMPB 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.[21] Olsen et al. reported the ability of estrogen to induce mACh-R binding in the central nerve system.[22] As a result, the loss of the ovarian function has a negative impact on basal forebrain cholinergic neurons.[23] A recent study suggested that women are at greater risk of Alzheimer's disease than men.[24] In our study, the slope of equations in the rNMPB ratio due to aging was found to be steeper in females than 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,[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 rNMPB 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.[15] Our results (12% higher rNMPB 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 (Rootwelts et al. reported 9% higher cerebellar blood flow in women[26]). A prior 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.[27] As a result, the decline in the rNMPB 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.[28] Their result (approximately 0.2% per year) was smaller than the decline in the rNMPB 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.[29] Their result was in contrast to our result indicating a steeper decline in the parietal and occipital rNMPB 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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## REFERENCES

1. Bartus RT, Dean RL III, Beer B, Lippa AS. The cholinergic hypothesis of geriatric memory dysfunction. *Science* 217: 408–417, 1982.
2. Mulholland GK, Kilbourn MR, Sherman P, Carey JE, Frey KA, Koeppe RA, et al. Synthesis, *in vivo* biodistribution and dosimetry of [$^{11}$C]*N*-methylpiperidyl benzilate ([$^{11}$C]NMPB), a muscarinic acetylcholine receptor antagonist. *Nucl Med Biol* 22: 13–17, 1995.
3. Mulholland GK, Jewett DM, Otto CA, Kilbourn MR, Sherman PS, Kuhl DE. Synthesis and regional brain distribution of [$^{11}$C]*N*-methyl-4-piperidyl benzilate ([$^{11}$C]NMPB) in the rat. *J Nucl Med* 29: 768, 1988. (Abstract)
4. Koeppe RA, Frey KA, Zubieta JA, Fessler JA, Mulholland GK, Kilbourn MR, et al. Tracer kinetic analysis of [$^{11}$C]*N*-methyl-4-piperidyl benzilate binding to muscarinic cholinergic receptors. *J Nucl Med* 33: 882, 1992. (Abstract)
5. Folstein MF, Folstein SE, McHugh PR. "Mini mental state." A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 12: 189–198, 1975.
6. Hasegawa K, Inoue K, Moriya K. An investigation of dementia rating scale for the elderly. *Seishinigaku* 16: 965–969, 1974. (Japanese)

7. Suhara T, Inoue O, Kobayashi K, Suzuki K, Tateno Y. Age-related changes in human muscarinic acetylcholine receptors measured by positron emission tomography. *Neurosci Lett* 149: 225–228, 1993.
8. Wong DF, Wagner HN Jr, Dannals RF, Links JM, Frost JJ, Ravert HT, et al. Effects of age on dopamine and serotonin receptors measured by positron tomography in the living human brain. *Science* 226: 1393–1399, 1984.
9. Yoshida T, Kuwabara Y, Ichiya Y, Sasaki M, Fukumura T, Ichimiya A, et al. Cerebral muscarinic acetylcholinergic receptor measurement in Alzheimer's disease patients on $^{11}$C-*N*-methyl-4-piperidyl benzilate—comparison with cerebral blood flow and cerebral glucose metabolism—. *Ann Nucl Med* 12: 35–42, 1998.
10. Dixon WJ, Brown MB, Engelman L, Jennrich RI. *BMDP Statistical Software Manual Vol. 2.* University of California Press, pp. 1122–1125, 1990.
11. White P, Hiley CR, Goodhardt MJ, Carrasco LH, Keet JP, Williams IE, et al. Neocortical cholinergic neurons in elderly people. *Lancet* 26: 668–671, 1977.
12. Rinne JO. Muscarinic and dopaminergic receptors in the aging human brain. *Brain Res* 404: 162–168, 1987.
13. Dewey SL, Volkow ND, Logan J, MacGregor RR, Fowler JS, Schlyer DJ, et al. Age-related decreases in muscarinic cholinergic receptor binding in the human brain measured with positron emission tomography (PET). *J Neurosci Res* 27: 569–575, 1990.
14. Lee KS, Frey KA, Koeppe RA, Buck A, Mulholland GK, Kuhl DE. *In vivo* quantification of cerebral muscarinic receptors in normal human aging using positron emission tomography and [$^{11}$C]tropanyl benzilate. *J Cereb Blood Flow Metab* 16: 303–310, 1996.
15. Rodriguez G, Warkentin S, Risberg J, Rosadini G. Sex differences in regional cerebral blood flow. *J Cereb Blood Flow Metab* 8: 783–789, 1988.
16. Andreason PJ, Zametkin AJ, Guo AC, Baldwin P, Cohen RM. Gender-related differences in regional cerebral glucose metabolism in normal volunteers. *Psychiatry Res* 51: 175–183, 1994.
17. Volkow ND, Wang GJ, Fowler JS, Hitzemann R, Pappas N, Pascani K, et al. Gender differences in cerebral metabolism: test-retest reproducibility. *Am J Psychiatry* 154: 119–121, 1997.
18. Gur RC, Mozley LH, Mozley PD, Resnick SM, Karp JS, Alavi A, et al. Sex differences in regional cerebral glucose metabolism during a resting state. *Science* 267: 528–531, 1995.
19. Baxter LR Jr, Mazziotta JC, Phelps ME, Selin CE, Guze BH, Fairbanks L. Cerebral glucose metabolic rates in normal human females versus normal males. *Psychiatry Res* 21: 237–245, 1987.
20. Hatazawa J, Brooks RA, Di Chiro G, Bacharach SL. Glucose metabolic rate versus brain size in humans. *J Cereb Blood Flow Metab* 7 (suppl 1): S301, 1987.
21. van Huizen F, March D, Cynader MS, Shaw C. Muscarinic receptor characteristics and regulation in rat cerebral cortex: changes during development, aging and the oestrous cycle. *Eur J Neurosci* 6: 237–243, 1994.
22. Olsen KL, Edwards E, Schechter N, Whalen RE. Muscarinic receptors in preoptic area and hypothalamus: effects of cyclicity, sex and estrogen treatment. *Brain Res* 448: 223–229, 1988.
23. Gibbs RB. Impairment of basal forebrain cholinergic neurons associated with aging and long-term loss of ovarian function. *Exp Neurol* 151: 289–302, 1998.
24. Henderson VW. The epidemiology of estrogen replacement therapy and Alzheimer's disease. *Neurology* 48 (Suppl 7): S27–S35, 1997.
25. Koeppe RA, Frey KA, Mulholland GK, Kilbourn MR, Buck A, Lee KS, et al. [$^{11}$C]tropanyl benzilate-binding to muscarinic cholinergic receptors: methodology and kinetic modeling alternatives. *J Cereb Blood Flow Metab* 14: 85–99, 1994.
26. Rootwelt K, Dybevold S, Nyberg-Hansen R, Russel D. Measurement of cerebral blood flow with $^{133}$Xe inhalation and dynamic single photon emission computer tomography. Normal values. *Scand J Clin Lab Invest Suppl* 184: 97–105, 1986.
27. Leenders KL, Perani D, Lammertsma AA, Heather JD, Buckingham P, Healy MJR, et al. Cerebral blood flow, blood volume and oxygen utilization, normal values and effect of age. *Brain* 113: 27–47, 1990.
28. Gur RC, Mozley PD, Resnick SM, Gottlieb GL, Kohn M, Zimmerman R, et al. Gender differences in age effect on brain atrophy measured by magnetic resonance imaging. *Proc Natl Acad Sci USA* 88: 2845–2849, 1991.
29. Coffey CE, Lucke JF, Saxton JA, Ratcliff G, Unitas LJ, Billig B, et al. Sex differences in brain aging—a quantitative magnetic resonance imaging study. *Arch Neurol* 55: 169–179, 1998.