Annals of Nuclear Medicine Vol. 17, No. 1, 73–77, 2003

Age-related changes of the binding of [^{3}H]SA4503 to sigma$_1$ receptors in the rat brain

Kiichi ISHIWATA,* Tadayuki KOBAYASHI,** Kazunori KAWAMURA*,*** and Kiyoshi MATSUNO****

*Positron Medical Center, Tokyo Metropolitan Institute of Gerontology
**M's Science Co.
***SHI Accelerator Service Co. Ltd.
****Nara Research and Development Center, Santen Pharmaceutical Co. Ltd.

We have recently developed 1-([3-*O*-methyl-^{11}C]3,4-dimethoxyphenethyl)-4-(3-phenylpropyl) piperazine ([^{11}C]SA4503) as a selective radioligand for mapping sigma$_1$ receptors in the brain by positron emission tomography (PET). In the present short communication we evaluated the age-related changes of the binding of this ligand to sigma$_1$ receptors in Fisher-344 rats (1.5-, 6-, 12-, and 24-month-old) by the *in vitro* binding assay. We also measured the binding of [^{3}H](+)-pentazocine to sigma$_1$ receptors and the binding of [^{3}H]1,3-di-*O*-tolylguanidine to sigma$_2$ receptors, which are current standard methods. The specific binding of the three radioligands increased age-dependently. Both K_d and B_{max} values of the 24-month-old rats for each radioligand were significantly higher than those of the young rats (1.5- and 6-month-old). The increased numbers of both sigma$_1$ and sigma$_2$ receptor subtypes in the aged rats compensate for the lowered affinity, and rather enhanced the radioligand-receptor binding. The results contrast strikingly with the age-dependent decrease in the dopaminergic, cholinergic and glutamatergic receptors that are reported to be correlated with the sigma receptors, and indicate that a PET study with [^{11}C]SA4503 to evaluate the aging process in humans would be of great interest.

Key words: [^{11}C]SA4503, sigma$_1$ receptor, aging, rat

INTRODUCTION

THE SIGMA RECEPTORS in the central nervous system are implicated in psychoses and movement disorders,[1,2] and at least two subtypes, sigma$_1$ and sigma$_2$ receptors modulate responses to several neuroreceptor functions such as dopaminergic, cholinergic and glutamatergic functions.[3–11] For the purpose of measurement of sigma receptors by positron emission tomography (PET), we have recently developed 1-([3-*O*-methyl-^{11}C]3,4-dimethoxyphenethyl)-4-(3-phenylpropyl)piperazine ([^{11}C]SA4503) as a selective PET ligand for sigma$_1$ receptors.[12–15] Preliminarily, we have successfully performed imaging of the sigma$_1$ receptors in the human brain by PET with [^{11}C]SA4503.[16,17] In general, the neuroreceptor system decreases with aging, and PET is a very powerful technique for investigation of the aging process in humans.[18–22] When we applied [^{11}C]SA4503 to studying the aging-related change of sigma$_1$ receptors in the monkey brain by PET, we found an age-related increase of the binding potential for [^{11}C]SA4503.[23] This *in vivo* binding parameter includes both the affinity (dissociation constant, K_d) and the maximal number of binding sites (B_{max}) of receptors.

In the present study, we investigated the age-related changes of the binding of [^{3}H]SA4503 to sigma$_1$ receptors in Fisher-344 rats. As for age-related changes in sigma receptors, Wallace et al. found a higher density of sigma receptor binding sites for nonselective [^{3}H]1,3-di-*O*-tolylguanidine ([^{3}H]DTG) in aged rats (37-month-old) than in young rats (4-month-old); however, they did not evaluate separately K_d and B_{max} values.[24] On the other hand, in postnatal developmental studies, Majewska et al.

Received September 21, 2002, revision accepted December 16, 2002.

For reprint contact: Kiichi Ishiwata, Ph.D., Positron Medical Center, Tokyo Metropolitan Institute of Gerontology, 1–1 Nakacho, Itabashi-ku, Tokyo 173–0022, JAPAN.

E-mail: ishiwata@pet.tmig.or.jp.

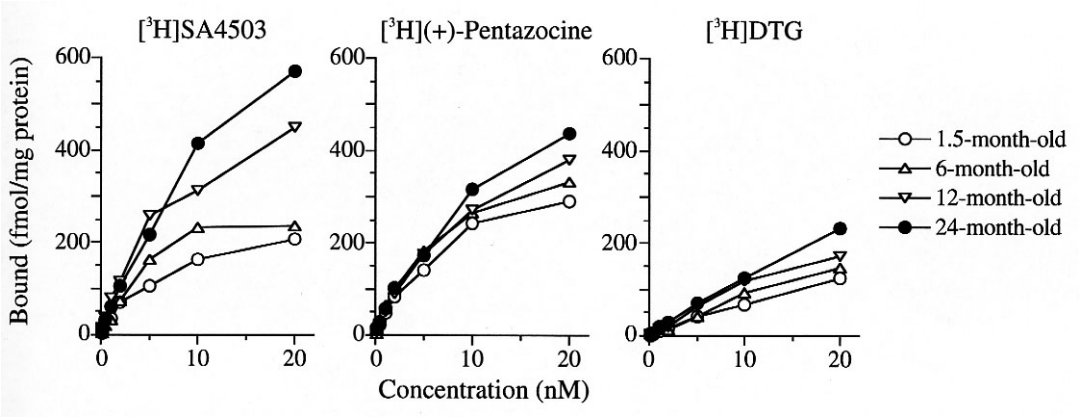

Fig. 1 Saturation curves for the specific bound of the each radioligands in the rat brain homogenate.

Table 1 Binding parameters calculated from saturation experiment with three radioligands

	Age (month)			
	1.5	6	12	24
	K_d (nM)			
[^{3}H]SA4503	4.5 ± 0.1	9.5 ± 0.6	5.8 ± 0.6	16.9 ± 0.9**,##
[^{3}H](+)-pentazocine	7.7 ± 0.4	6.8 ± 0.2	9.8 ± 1.0	9.7 ± 0.8#
[^{3}H]DTG	10.8 ± 0.0	8.3 ± 0.0	15.2 ± 2.4	25.4 ± 0.4
	B_{max} (fmol/mg protein)			
[^{3}H]SA4503	234 ± 4	421 ± 24	545 ± 41	1069 ± 56**,##
[^{3}H](+)-pentazocine	404 ± 15	449 ± 12	554 ± 47	622 ± 28**,##
[^{3}H]DTG	162 ± 2	176 ± 1	204 ± 15	483 ± 6**,##

Data given are the means ± S.E. of three determinations. Dunnett's multiple-range test was performed between 1.5- and 24-month-old rats and between 6- and 24-month-old rats. **$p < 0.01$ vs. 1.5 month-old group; and #$p < 0.05$ and ##$p < 0.01$ vs. 6 month-old group.

reported that both the affinity and density of sigma sites in the rat brain remained constant throughout postnatal day 1 to 1 year,[25] while Matsumoto et al. described that middle-aged rats (5–6 months old) had fewer sigma binding sites with lower affinity for [^{3}H]DTG than younger adult rats (2- to 3-month-old).[26] All the three groups measured both sigma$_1$ and sigma$_2$ receptor subtypes together using nonselective [^{3}H]DTG or [^{3}H]haloperidol in the presence of spiperone to block ligand binding to dopamine D_2 receptors.[24–26] Therefore, in the present study, we measured the binding parameters of two sigma receptor subtypes separately. For the assay of sigma$_1$ receptors we used not only [^{3}H]SA4503 but also [^{3}H](+)-pentazocine, because the competition *in vivo* in the binding of [^{11}C]SA4503 and (+)-pentazocine to the sigma$_1$ binding sites apparently differs from their competition *in vitro*.[12] For the assay of sigma$_2$ receptors [^{3}H]DTG was used in the presence of (+)-pentazocine to block sigma$_1$ binding sites. The use of [^{3}H](+)-pentazocine and [^{3}H]DTG in the presence of (+)-pentazocine is a now standard method for measuring *in vitro* sigma$_1$ and sigma$_2$ receptors, respectively.

MATERIALS AND METHODS

SA4503 and (+)-pentazocine were synthesized at Santen Pharmaceutical Co. Ltd., (Osaka, Japan). 1,3-di-*O*-tolylguanidine (DTG) was purchased from Research Biochemicals International (Natick, MA, USA). [^{3}H]SA4503 was prepared by methylation of the respective demethyl compound with [^{3}H]methyl iodide at Amersham Pharmacia Biotech (Buckinghamshire, England) as described.[12] [^{3}H](+)-pentazocine and [^{3}H]DTG were obtained from NEN^{TM} Life Science Products (Boston, MA, USA).

Six-week-old and 6-, 12- and 24-month-old virgin male Fischer-344 rats were supplied from the Department of Laboratory Animal Science at the Tokyo Metropolitan Institute of Gerontology. They were maintained in an air-conditioned and light controlled environment (22°C, 12 h light and 12 h dark), with water and food ad libitum. The animal studies were approved by the Animal Care and Use Committee of the Tokyo Metropolitan Institute of Gerontology.

Binding of [^{3}H]S4503, [^{3}H](+)-pentazocine and [^{3}H]DTG to the sigma receptors

Six rats of each of four groups were killed by cervical dislocation, and membrane preparations from the whole brain were prepared as described previously.[27] The binding of [^{3}H]SA4503 or [^{3}H](+)-pentazocine to the sigma$_1$ receptors and the binding of [^{3}H]DTG to sigma$_2$ receptors in the presence of 200 nM (+)-pentazocine were determined as previously described.[27] Specific binding was determined by subtracting the binding in the presence of each unlabeled ligand from the binding in its absence. Each assay was conducted in triplicate, and each experiment was repeated three times. The results are expressed as mean ± S.E. The values of K_d and B_{max} were calculated by linear-squares regression analysis. The statistical significance of differences between each group was determined by analysis of variance followed by Dunnett's multiple range comparison test.

RESULTS

Figure 1 shows the saturation analysis of each of the three radioligands at each age. The specific binding increased with age. The data on K_d and B_{max} from the binding assay with each of the three radioligands are summarized in Table 1. The K_d values of the 24-month-old rats for each radioligand were larger than those of the 1.5- and 6-month-old rats: 3.7 and 1.8 fold for [^{3}H]SA4503, 1.3 (not significant) and 1.4 fold for [^{3}H](+)-pentazocine and 3.0 and 2.8 fold for [^{3}H]DTG. A similar age-dependent increase was found for the B_{max} values: 4.6 and 2.5 times for [^{3}H]SA4503, 1.5 and 1.4 times for [^{3}H](+)-pentazocine, and 3.0 and 2.8 times for [^{3}H]DTG being significantly affected by aging.

DISCUSSION

Recently, we found an age-related increase in the binding potential for [^{11}C]SA4503 in the monkey brain by PET.[23] In the present study we found a similar age-related increase in the [^{3}H]SA4503 binding in the rat brain by the *in vitro* membrane binding assay. The specific binding of all three radioligands increased age-dependently (Fig. 1). The affinity (K_d) was weaker in the 24-month-old rats than in the 1.5- and 6-month-old rats, whereas the receptor density (B_{max}) increased age-dependently. The same phenomena were also found for the binding of [^{3}H](+)-pentazocine to the sigma$_1$ receptors, and for the binding of [^{3}H]DTG to the sigma$_2$ receptors. The increase in the binding sites in the aged rats compensates for the weaker affinity for both sigma$_1$ and sigma$_2$ receptor subtypes, and rather enhanced the radioligand-receptor binding.

As for the sigma$_1$ receptors, the numbers of the sigma$_1$ binding sites (B_{max}) for [^{3}H]SA4503 were more than those for [^{3}H](+)-pentazocine in the 24-month-old rats, although they were comparable in the three other groups. A possible explanation for this is that the selectivity of the two compounds for sigma receptor subtypes is different. SA4503 was developed by Matsuno et al. as a sigma$_1$ receptor agonist having a comparable affinity but a slightly less selectivity than (+)-pentazocine.[27] The IC_{50} values of SA4503 are 17.4 nM and 1,784 nM for sigma$_1$ and sigma$_2$ receptor subtypes, respectively, (sigma$_1$/sigma$_2$ selectivity = 103), whereas the corresponding values of (+)-pentazocine are 13.7 nM and 2,875 nM (sigma$_1$/sigma$_2$ selectivity = 210).[27] Thereby, the binding parameters of [^{3}H]SA4503 for sigma receptors might be affected to a certain extent by the age-related change in the sigma$_2$ receptor subtype. As another possibility, minor affinities of μM order of the two ligands for other binding sites may explain the difference between [^{3}H]SA4503 binding and [^{3}H](+)-pentazocine binding; i.e. the affinity of (+)-pentazocine for PCP sites[28] and the affinity of SA4503 for α_1 adrenoceptors[27] and for dopamine D_2 receptors.[14] Bowen et al. (1989) hypothesized an allosteric model of sigma-binding sites and suggested that sigma ligands may interact with either a neuroleptic binding site or (+)-benzomorphan-binding site on the sigma receptor macromolecule.[29] Therefore, an other explanation is that the structural difference between (+)-pentazocine and SA4503 affects the sigma$_1$ receptor binding in the aged rat. That is, the structure of SA4503, a piperazine compound, may be more sensitive to sigma$_1$ receptor binding than that of (+)-pentazocine having a benzomorphane structure due to age-related change.

On the other hand, the age-related decrease in the dopaminergic, cholinergic and glutamatergic receptors is well known in experimental animals[8,30–32] and in humans.[18–22] In addition, sigma receptors interact with these neural transmission systems. For example, sigma receptor ligands affected the extracellular concentration of dopamine in the rat brain[3,5,9] and sigma$_1$ receptor agonists increased dose-dependently the extracellular level of acetylcholine in the rat brain.[6,10] Therefore, our data suggest that the increase in the sigma receptor density might couple with the decrease in the activity of the dopaminergic, cholinergic and glutamatergic neurons with increasing age, like the age-related concurrent changes in dopamine D_2 and sigma receptors suggested by Wallace et al.[24] In other words, the sigma receptors might be increased to compensate for the decline of the function at the dopaminergic, cholinergic and glutamatergic neurons. As another possibility, the affinity (K_d) and the density (B_{max}) may be related to each other, with the numbers of sigma receptors increased to support the loss of the receptor function due to the lower affinity of those receptors with aging.

In the present study we used membrane preparation of whole brain, because *in vitro* autoradiography[33] with [^{3}H](+)-pentazocine and [^{3}H]DTG and *ex vivo* autoradiography[12] with [^{11}C]SA4503 showed widely distributed binding sites in the brain. However, the autoradiographic

evaluation make possible delineation of regional differences in age-related changes, which would help to make clear the physiological significance of the sigma receptors.

As for the age-related change of the sigma receptors in the human brain, Kornhuber et al. found an age-related decrease in the binding of [^{3}H](+)-pentazocine to the sigma$_1$ receptors in post-mortem human frontal cortex.[34] They reported that the sigma$_1$ receptors in the human cortex were not changed during storage for up to six years; however, we preliminarily found that the storage of the brain tissues of guinea pigs, not of membrane preparation, decreased the sigma receptor binding compared with freshly prepared membrane preparation. The different procedures for membrane preparations might result in difference in the age-related changes between the rat brain and the post-mortem human brain.

In conclusion, we found an age-related increase in the binding of radioligands to both sigma$_1$ and sigma$_2$ receptors in the rat brain by the membrane binding assay. Together with further evaluation of the physiological significance of the sigma receptors in the brain, a PET study with [^{11}C]SA4503 to evaluate the aging process in humans would be of great interest, and is in progress.

ACKNOWLEDGMENT

This work was supported by a Grant-in-Aid for Scientific Research (B) No. 13557077 from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

REFERENCES

1. Junien JL, Leonard BE. Drugs acting on sigma and phencyclidine receptors: A review of their nature, function and possible therapeutic importance. *Clin Neuropharmacol* 1989; 12: 353–374.
2. Su TP. Delineating biochemical and functional properties of sigma receptors: emerging concepts. *Crit Rev Neurobiol* 1993; 7: 187–203.
3. Patrick SL, Walker JM, Perkel JM, Lockwood M, Patrick RL. Increases in rat striatal extracellular dopamine and vacuous chewing produced by two sigma receptor ligands. *Eur J Pharmacol* 1993; 231: 243–249.
4. Matsuno K, Senda T, Kobayashi T, Mita S. Involvement of σ_1 receptor in (+)-*N*-allynormetazocine—stimulated hippocampal colinergic function in rats. *Brain Res* 1995; 690: 200–206.
5. Gudelsky GA. Effects of σ receptor ligands on the extracellular concentration of dopamine in the striatum and prefrontal cortex of the rat. *Eur J Pharmacol* 1995; 286: 223–228.
6. Kobayashi T, Matsuno K, Mita S. Regional differences of the effect of σ receptor ligands acetylcholine release in the rat brain. *J Neural Transm* 1996; 103: 661–669.
7. Kobayashi T, Matsuno K, Murai M, Mita S. σ_1 receptor subtype is involved in the facilitation of cortical dopaminergic transmission in the rat brain. *Neurochem Res* 1997; 22: 1105–1109.
8. Buyukuysal RL, Ulus I, Kiran BK. Age-related alterations in pre-synaptic and receptor-mediated cholinergic function in rat brain. *Neurochem Res* 1998; 23: 719–726.
9. Gudelsky GA. Biphasic effect of sigma receptor ligands on the extracellular concentration of dopamine in the striatum of the rat. *J Neural Transm* 1999; 106: 849–856.
10. Siniscalchi A, Cristofori P, Veratti E. Influence of *N*-allylnormetazocine on acetylcholine release from brain slices: involvement muscarinic receptors. *Naunyn-Schmeideberg's Arch Pharmacol* 1987; 336: 425–429.
11. Walker JM, Bowen WD, Walker FO, Matsumoto RR, de Costa B, Rice KC. Sigma receptors: biology and function. *Pharmacol Rev* 1990; 42: 355–402.
12. Kawamura K, Ishiwata K, Tajima H, Ishii S, Matsuno K, Homma Y, et al. *In vivo* evaluation of [^{11}C]SA4503 as a PET ligand for mapping CNS sigma$_1$ receptors. *Nucl Med Biol* 2000; 27: 255–261.
13. Kawamura K, Ishiwata K, Shimada Y, Kimura Y, Kobayashi T, Matsuno K, et al. Preclinical evaluation of [^{11}C]SA4503: radiation dosimetry, *in vivo* selectivity and PET imaging of sigma$_1$ receptors in the cat brain. *Ann Nucl Med* 2000; 14: 285–292.
14. Ishiwata K, Kobayashi T, Kawamura K, Matsuno K, Senda M. [^{11}C]Raclopride binding was reduced *in vivo* by sigma$_1$ receptor ligand SA4503 in the mouse brain, while [^{11}C]SA4503 binding was not by raclopride. *Nucl Med Biol* 2001; 28: 787–792.
15. Ishiwata K, Tsukada H, Kawamura K, Kimura Y, Nishiyama S, Kobayashi T, et al. Mapping of CNS sigma$_1$ receptors in the conscious monkey: a preliminary PET study with [^{11}C]SA4503. *Synapse* 2001; 40: 235–237.
16. Ishii K, Ishiwata K, Kimura Y, Kawamura K, Oda K, Senda M. Mapping of sigma$_1$ receptors in living human brain. *Neuroimage* 2001; 6 (Part 2 of 2 parts): S984.
17. Ohyama N, Ishiwata K, Ishii K, Mishina M, Kitamura S, Kawamura K, et al. The first demonstration of sigma$_1$ receptors with Alzheimer's disease using PET and a newly developed ligand: ^{11}C-SA4503, *J Nucl Med* 43; 2002: 243P.
18. 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* 1990; 27: 569–575.
19. Rinne JO, Hietala J, Ruotsalainen U, Sako E, Laihinen A, Nagren K, et al. Decrease in human striatal dopamine D2 receptor density with age: a PET study with [^{11}C]raclopride. *J Cereb Blood Flow Metab* 1993; 13: 310–314.
20. Wang Y, Chan GL, Holden JE, Dobko T, Mak E, Schulzer M, et al. Age-dependent decline of dopamine D1 receptors in human brain: a PET study. *Synapse* 1998; 30: 56–61.
21. Meng SZ, Ozawa Y, Itoh M, Takashima S. Developmental and age-related change of dopamine transporter, and dopamine D1 and D2 receptors in human basal ganglia. *Brain Res* 1999; 843: 136–144.
22. Kaasien V, Vilkman H, Hietala J, Någren K, Helenius H, Olssen H, et al. Age-related dopamine D2/D3 receptor loss in extrastriatal regions of the human brain. *Neurobiol Aging* 2000; 21: 683–688.
23. Kawamura K, Kimura Y, Tsukada H, Kobayashi T, Nishiyama S, Kakiuchi T, et al. An increase of sigma$_1$ receptors in the aged monkey brain. *Neurobiol Aging* (in

press).
24. Wallace DR, Mactutus CF, Booze RM. Sigma binding sites identified by [^{3}H]DTG are elevated in aged Fischer-344 x Brown Norway (F1) rats. *Synapse* 2000; 35: 311–313.
25. Majewska MD, Parameswaran S, Vu T, London ED. Divergent ontogeny of sigma and phencyclidine binding sites in the rat brain. *Brain Res Dev Brain Res* 1989; 47: 13–18.
26. Matsumoto RR, Bowen WD, Walker JM. Age-related differences in the sensitivity of rats to a selective sigma ligand. *Brain Res* 1989; 504: 145–148.
27. Matsuno K, Nakazawa M, Okamoto K, Kawashima Y, Mita S. Binding properties of SA4503 a novel and selective σ_1 receptor agonist. *Eur J Pharmacol* 1996; 306: 271–279.
28. Whittemore ER, Ilyin VI, Woodward RM. Antagonism of *N*-methyl-D-aspartate receptors by a site ligands: potency, subtype-selectivity and mechanisms of inhibition. *J Pharmacol Exp Ther* 1997; 282: 326–338.
29. Bowen WD, Hellewell SB, McGrarry KA. Evidence for a multisite model of the rat brain σ receptor. *Eur J Pharmacol* 1989; 163: 309–318.
30. Giardino L. Right-left asymmetry of D1- and D2-receptor density is lost in the basal ganglia of old rats. *Brain Res* 1996; 720: 235–238.
31. Muller WE, Stoll S, Scheuer K, Meichelbock A. The function of the NMDA-receptor during normal brain aging. *J Neural Transm Suppl* 1994; 44: 145–158.
32. Smith TD, Gllagher M, Lesline FM. Cholinergic binding sites in rat brain: analysis by age and cognitive status. *Neurobiol Aging* 1995; 16: 161–173.
33. Bouchard P, Quirion R. [^{3}H]1,3-di(2-tolyl)guanidine and [^{3}H](+)pentazocine binding sites in the rat brain: autoradiographic visualization of the putative sigma$_1$ and sigma$_2$ receptor subtypes. *Neuroscience* 1997; 76: 467–477.
34. Kornhuber J, Schoppmeyer K, Bendig C, Riederer P. Characterization of [^{3}H]-pentazocine binding sites in post-mortem human frontal cortex. *J Neural Transm* 1996; 103: 45–53.