Effects of *Kamikihito*, a Traditional Chinese Medicine, on Neurotransmitter Receptor Binding in the Aged Rat Brain Determined by In Vitro Autoradiography (1): Changes in the [^{3}H]QNB Binding

Tetsuo Hayashi[1], Kiyofumi Yamada[1], Takaaki Hasegawa[1], Seiichi Ishihara[2], Tsutomu Kameyama[2], Tadaomi Morimasa[3], Takao Kaneyuki[3], Toshikiyo Shohmori[3] and Toshitaka Nabeshima[1,*]

[1]*Department of Neuropsychopharmacology and Hospital Pharmacy, Nagoya University School of Medicine, Nagoya 466, Japan*
[2]*Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Meijo University, Nagoya 468, Japan*
[3]*Department of Neurology, Okayama University Medical School, Okayama 700, Japan*

Received December 7, 1993 Accepted February 18, 1994

ABSTRACT—Using in vitro autoradiography, we investigated the effects of *Kamikihito* (KKT), a traditional Chinese medicine, on the specific binding of [^{3}H]quinuclidinyl benzilate (QNB) and [^{3}H]*N*-(1-[2-thienyl]cyclohexyl)-3,4-piperidine (TCP) in the rat brain. The B_{max} but not the K_d values for [^{3}H]QNB binding to the caudate/putamen and accumbens in aged rats were lower than those in young rats. The [^{3}H]TCP binding was also decreased in aged rats compared with that in young rats. Long-term administration of KKT modulated the [^{3}H]QNB binding in young but not aged rats.

Keywords: *Kamikihito*, [^{3}H]QNB binding, [^{3}H]TCP binding

Kamikihito (KKT), which consists of Astragalus, Ginseng, Atractylodes, Hoelen, Polygala, Jujube, Longan, Zizyphus, Angelica, Licorice, Ginger, Saussurea, Bupleurum and Gardenia, is a traditional Chinese medicine that is used to treat anemia, insomnia anxiety and neurosis. Recently, it has been demonstrated that KKT improves learning performance in the senescent accelerated mouse (SAM) (1). Furthermore, it has been reported that the cognitive ability of alcoholic patients was improved by KKT (2). These studies suggest that KKT may ameliorate memory impairment in senile dementia.

In previous studies, we investigated learning and memory and emotional behavior in aged rats (3-5), finding that not only learning and memory but also the emotional behavior was impaired in these animals. Since psychotic symptoms, including anxiety, depression, delusions and hallucinations, frequently accompany memory impairment in the elderly (6), our previous results suggest that aged rats are useful as a senile dementia model.

In this study, by using in vitro quantitative autoradiography, we measured the specific binding of [^{3}H]-quinuclidinyl benzilate (QNB) and [^{3}H]*N*-(1-[2-thienyl]-cyclohexyl)-3,4-piperidine (TCP) in the brains of aged rats. We also investigated the effects of long-term administration of KKT on the binding of these receptors.

The aged animals used were male Fischer rats (99 weeks old before the experiment; Charles River Japan, Inc., Hino, Shiga). Young control rats were 6 weeks old before the experiment. All animals were kept in a temperature- and light-controlled room (23℃, 12-hr light cycle starting at 9:00 a.m.). The animals were given a regular diet or one containing KKT (8%; Kanebo Co., Ltd., Tokyo) for 15 weeks. They were then sacrificed for autoradiography, at which time the aged and young rats were 114 and 21 weeks old, respectively. The calculated daily dose of KKT in young and aged rats was 1.25 and 1.36 g/rat, respectively. The body weights of aged rats given the regular and KKT-containing diets after the 15-week period of drug administration were 484 ± 4 and 461 ± 5 g, respectively, while those of young rats given the regular and KKT-containing diets were 287 ± 17 and 278 ± 15 g, respectively. Rats were sacrificed by decapitation, and their brains were rapidly frozen at -100℃. Twenty-μm cryostat sections were prepared for the binding assay. Autoradiography of [^{3}H]QNB (0.05-2.0 nM; specific activity, 44.3 Ci/mmol; NEN Research Products, Boston,

*To whom correspondence should be addressed.

MA, USA) and [^{3}H]TCP (20 nM; specific activity, 48.9 Ci/mmol; NEN Research Products) was carried out as described previously (7). Non-specific binding of [^{3}H]-QNB and [^{3}H]TCP was defined as the binding in the presence of 20 μM atropine (Sigma, St. Louis, MO, USA) and 20 μM phencyclidine (PCP, synthesized by Prof. Dr. H. Furukawa, Meijo University), respectively. The specific binding of [^{3}H]QNB and [^{3}H]TCP represented approximately 85% and 95% of the total binding, respectively. Results were expressed as means±S.E. (n=5). The significance of differences was assessed by the two-tailed Student's *t*-test.

The results of Scatchard analysis of [^{3}H]QNB binding to different brain regions in young and aged rats are shown in Table 1. The maximal number of binding sites (B_{max}) for [^{3}H]QNB in the caudate/putamen and accumbens of aged rats was significantly lower than that in young rats, while its affinity for muscarinic cholinergic (m-ACh) receptors remained unchanged. There were no differences in other brain regions, including the cortex, the CA1 subfield of the hippocampus, the thalamus, hypothalamus and amygdala, between the two groups. The B_{max} in the caudate/putamen and accumbens and the K_d value in the cortex and caudate/putamen for [^{3}H]QNB binding in young rats given the KKT-containing diet (KKT-young rats) was significantly decreased, compared with these values for young rats given the regular diet (r-young rats). The K_d value for [^{3}H]QNB binding to the CA1 subfield of the hippocampus in aged rats given the KKT-containing diet (KKT-aged rats) was decreased compared with that in aged rats given the regular diet (r-aged rats), although the changes were not significant (P=0.052). When the binding in the whole hippocampus was compared, the difference between r-aged and KKT-aged rats was less pronounced (data not shown). In other brain regions, there were no differences in the parameters of [^{3}H]QNB binding between r-aged and KKT-aged rats (Table 1).

The specific binding of [^{3}H]TCP (20 nM) to ion channels (PCP binding sites) coupled with *N*-methyl-D-aspartate (NMDA) receptors in the brains of young and aged rats is shown in Table 2. There was a significant decrease in specific [^{3}H]TCP binding to the caudate/putamen (−38.6%) and hippocampus (−13.5%) in aged rats compared with that in young rats. In KKT-young rats, the [^{3}H]TCP binding to the caudate/putamen decreased, while the binding to the hypothalamus increased, compared with these values in r-young rats. There were no differences in [^{3}H]TCP binding between KKT-aged and r-aged rats in any of the examined brain regions (Table 2).

There was a small but significant reduction in the B_{max}, but not K_d values, of [^{3}H]QNB binding to the caudate/putamen (−9.5%) and accumbens (−7.2%) in aged rats compared with these values in young rats. In previous studies, it has also been shown that the age-related reduction in m-ACh receptor binding appears to be small and inconsistent (for reviews, see ref. 8).

The specific binding of [^{3}H]TCP to PCP binding sites coupled with NMDA receptors was significantly

Table 1. Scatchard analysis of [^{3}H]QNB binding in the brains of young and aged rats given regular or KKT (8%)-containing diet for 15 weeks

Brain region	B_{max} (fmol/mg protein)			
	r-young	KKT-young	r-aged	KKT-aged
Cortex	713±13	680±21	678±11	646±22
Caudate/putamen	792±19	717±18*	716±14*	672±28
Accumbens	781±15	727±15*	725±18*	688±21
Hippocampal CA1	780±11	794±28	810±25	755±24
Thalamus	535±18	585±66	502±24	475±14
Hypothalamus	464±15	458±37	430±10	426± 9
Amygdala	753±20	754±25	710±33	688±30

Brain region	K_d (pM)			
	r-young	KKT-young	r-aged	KKT-aged
Cortex	91± 8	65± 5*	79± 7	71± 7
Caudate/putamen	124±14	82± 7*	103±14	80± 9
Accumbens	94±10	72± 5	81±15	57± 4
Hippocampal CA1	66±13	72± 8	79± 5	58± 8
Thalamus	139±10	121±14	138±19	114±11
Hypothalamus	133±14	139±23	131±19	117±11
Amygdala	86± 7	80± 8	63±11	54± 7

r-young: young rats given regular diet; KKT-young: young rats given KKT-containing diet; r-aged: aged rats given regular diet; KKT-aged: aged rats given KKT-containing diet. Each value represents the mean±S.E. (n=5). *P<0.05 vs. r-young rats.

Table 2. Binding of [^{3}H]TCP in the brains of young and aged rats given regular or KKT (8%)-containing diet for 15 weeks

Brain region	r-young	KKT-young	r-aged	KKT-aged
Cortex	157±12	157± 6	134±14	138± 8
Caudate/putamen	125± 4	114± 3*	77±13**	87± 5
Accumbens	125± 8	128± 2	98±13	90± 7
Hippocampus	194±10	207± 9	168± 6*	184± 7
Thalamus	100±10	114± 2	79±11	93± 9
Hypothalamus	41±10	65± 4*	45±10	45±12
Amygdala	123±12	137± 4	126±13	103± 9

Specific [^{3}H]TCP (20 nM) binding is expressed as fmol/mg protein. r-young: young rats given regular diet; KKT-young: young rats given KKT-containing diet; r-aged: aged rats given regular diet; KKT-aged: aged rats given KKT-containing diet. Each value represents the mean±S.E. (n=5). *P<0.05, **P<0.01 vs. r-young rats.

decreased in the caudate/putamen and hippocampus in aged rats compared with that in young rats. Kito et al. (9) have demonstrated by in vitro autoradiography that the [^{3}H]glycine binding sites of the NMDA receptor/ionophore complex in the hippocampus and cortex were markedly (approximately −50%) decreased in aged rats, while [^{3}H]CPP binding to NMDA receptors was well preserved in these brain regions. Taken together, these results and other findings suggest that, within the NMDA receptor/ionophore complex, PCP binding sites, as well as glycine receptors, may be primarily affected during aging.

ACh and glutamate are neurotransmitters that play an important role in memory and learning. Scopolamine, a m-ACh receptor antagonist, impairs learning and memory in both humans and laboratory animals (10), while lesioning of cholinergic neurons in the basal forebrain produces impairments of learning and memory in rats (11). With regard to glutamate, it has been demonstrated that NMDA antagonists both inhibit long-term potentiation, a process that may underlie some forms of memory, and impair learning behavior in rats (12). Taking these previous results together with our present findings, it is conceivable that a decrease in the specific binding of [^{3}H]-QNB and [^{3}H]TCP in the brain may be responsible, at least in part, for the impairments of learning and memory that occur in aged rats (5, 13)

We have demonstrated here, using an in vitro quantitative autoradiographic technique, that long-term administration of KKT significantly decreased the B_{max} of [^{3}H]-QNB binding to the caudate/putamen and accumbens, and increased the affinity of [^{3}H]QNB for its receptor in the cortex and caudate/putamen of young rats. In contrast, KKT had little effect on [^{3}H]QNB binding in aged rats, although in aged rats, we have observed that KKT tended to increase the affinity of [^{3}H]QNB for its receptor, without changing the B_{max} in the CA1 subfield of the hippocampus. The relative insensitivity to KKT administration in aged rats may be due to the impaired regulation of receptor density in aged rats, as indicated by Pedigo and Polk (14). Egashira et al. (15) have demonstrated that long-term administration of KKT (200 mg/kg/day, p.o. for 4 weeks) increased the B_{max} of [^{3}H]QNB binding in membrane preparations of cortical regions from aged rats. Although there appears to be a difference in the direction of the changes in [^{3}H]QNB binding produced by KKT, our findings and those of Egashira et al. suggest that long-term administration of KKT affects the neurotransmission of ACh in the brains of both young and aged rats. The reasons for the difference in the changes in [^{3}H]QNB binding produced by KKT in the two studies are unclear. Differences in the strain of rats and methods utilized to determine the binding activity, as well as the different doses and periods of KKT administration, may be involved. We consider that the changes produced in [^{3}H]-QNB binding by the long-term administration of KKT reflect adaptive responses of the cholinergic receptors to KKT-induced continuous activation. Since the dysfunction of cholinergic neurons is considered to be an important factor in memory impairment in the elderly (11), it is notable that in aged rats, KKT showed a tendency to increase the affinity of [^{3}H]QNB binding without changing the B_{max} in the CA1 subfield of the hippocampus, this region being one of the most important brain structures in learning and memory. To elucidate the role of the cholinergic neuronal mechanism in the ameliorating effects of KKT on the cognitive impairment of alcoholic patients (2) and in the SAM (1), the effects of KKT on other cholinergic neuronal markers such as the neuronal response to cholinergic stimulation, ACh synthesis and release should be determined.

Long-term administration of KKT affected [^{3}H]TCP binding in the brains of young, but not aged rats, decreasing the binding to the caudate putamen, but increasing that to the hypothalamus. These results suggest that KKT may modulate [^{3}H]TCP binding in young but not aged rats. Further studies should be carried out to determine the effects of KKT on [^{3}H]TCP binding.

Acknowledgments

This study was supported, in part, by a SRF Grant for Biomedical Research and grants from the Ministry of Health and Welfare Foundation for Gerontological Science Research (91A-2406), Ministry of Education, Science and Culture, Japan for Science Research (No. 05454148) and the Japan Research Foundation for Clinical Pharmacology.

REFERENCES

1 Nishizawa K, Saito H and Nishiyama N: Effects of *Kamikihi-To*, a traditional Chinese medicine, on passive and conditioned avoidance performance impairment in senescence accelerated mouse (SAM). Jpn J Pharmacol **54**, 375–382 (1990)

2 Masuda Y, Mizuguchi S, Sugiyama K and Hishikawa Y: Effect of *Kamikihito* on cognitive dysfunction in alcoholic patients. Prog Med **10**, 1172–1174 (1990) (Abstr in English)

3 Ishimaru H, Ogawa S, Fuji K, Fukuta T, Kameyama T and Nabeshima T: Aged-related changes in learning and memory, choline acetyltransferase activity and number of neuronal cells in rats. J Pharmacobiodyn **14**, 321–325 (1991)

4 Nabeshima T, Hasegawa M, Nakayama S, Kinoshita H, Amano M and Hasegawa T: Impairment of learning and memory and the accessory symptom in aged rats as senile dementia model. (1) Emotional behavior. Jpn J Psychopharmacol **13**, 65–72 (1993) (Abstr in English)

5 Nabeshima T, Hasegawa M, Nakayama S, Kinoshita H, Amano M and Hasegawa T: Impairment of learning and memory and the accessory symptom in aged rats as senile dementia model. (2) Learning and memory. Jpn J Psychopharmacol **13**, 73–79 (1993) (Abstr in English)

6 Wragg RE and Jeste DV: Overview of depression and psychosis in Alzheimer's disease. Am J Psychiatry **146**, 577–587 (1989)
7 Murase K, Nabeshima T, Kameyama T, Sasa M, Takaori S, Ujihara H, Ishihara K, Yamada J and Serikawa T: Characteristics of muscarinic, cholinergic, γ-aminobutyric $acid_A$ and phencyclidine receptors in spontaneously epileptic rats; in vitro quantitative autoradiographic analysis. Neurosci Lett **131**, 1–4 (1991)
8 Decker MW: The effects of aging on hippocampal and cortical projections of the forebrain cholinergic system. Brain Res Rev **12**, 423–438 (1987)
9 Kito S, Miyoshi R and Nomoto T: Influence of age on NMDA receptor complex in rat brain studied by in vitro autoradiography. J Histochem Cytochem **38**, 1725–1731 (1990)
10 Bartus RT, Dean RL, Beer B and Lippa AS: The cholinergic hypothesis of geriatric memory dysfunction. Science **217**, 408–417 (1982)
11 Nabeshima T, Ogawa S, Nishimura H, Fuji K, Kameyama T and Sasaki Y: Staurosporine facilitates recovery from the basal forebrain lesion-induced impairment of learning and deficit of cholinergic neuron in rats. J Pharmacol Exp Ther **257**, 562–566 (1992)
12 Morris RGM, Anderson E, Lynch G and Baudry M: Selective impairment of learning and blockade of long term potentiation by an *N*-methyl-D-aspartate receptor antagonist, AP-5. Nature **319**, 774–776 (1986)
13 Gage FH, Dunnett SB and Bjorklund A: Spatial learning and motor deficits in aged rats. Neurobiol Aging **5**, 43–48 (1984)
14 Pedigo NW Jr and Polk DM: Reduced muscarinic receptor plasticity in frontal cortex of aged rats after chronic administration of cholinergic drugs. Life Sci **37**, 1443–1449 (1985)
15 Egashira T, Sudo S, Murayama F, Kono T, Kudo Y, Goto S, Takayama F and Yamanaka M: Effects of *kamikihi-to*, a Chinese traditional medicine, on various cholinergic biochemical markers in the brains of aged rats. Folia Pharmacol Jpn **98**, 273–281 (1991) (Abstr in English)