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 [3H]QNB Binding

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

Keywords: Kamikihito, [3H]QNB binding, [3H]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 [3H]-quinuclidinyl benzilate (QNB) and [3H]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°C, 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.

Rats were sacrificed by decapitation, and their brains were rapidly frozen at −100°C. Twenty-μm cryostat sections were prepared for the binding assay. Autoradiography of [3H]QNB (0.05–2.0 nM; specific activity, 44.3 Ci/mmol; NEN Research Products, Boston,
MA, USA) and [3H]TCP (20 nM; specific activity, 48.9 Ci/mmol; NEN Research Products) was carried out as described previously (7). Non-specific binding of [3H]QNB and [3H]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 [3H]QNB and [3H]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 [3H]QNB binding to different brain regions in young and aged rats are shown in Table 1. The maximal number of binding sites (Bmax) for [3H]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 Bmax in the caudate/putamen and accumbens and the Kd value in the cortex and caudate/putamen for [3H]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 Kd value for [3H]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 [3H]QNB binding between r-aged and KKT-aged rats (Table 1).

The specific binding of [3H]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 [3H]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 [3H]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 [3H]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 Bmax, but not Kd values, of [3H]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 [3H]TCP to PCP binding sites coupled with NMDA receptors was significantly

| Brain region         | Bmax (fmol/mg protein) | Kd (pM) |
|----------------------|------------------------|---------|
|                      | 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         | Kd (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 [3H]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 [3H]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 [3H]glycine binding sites of the NMDA receptor/ionophore complex in the hippocampus and cortex were markedly (approximately −50%) decreased in aged rats, while [3H]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 [3H]QNB and [3H]TCP binding to the cortex and caudate/putamen of young rats. In contrast, KKT had little effect on [3H]QNB binding in aged rats, although in aged rats, we have observed that KKT tended to increase the affinity of [3H]QNB for its receptor in the cortex and caudate/putamen of young rats. Further studies should be carried out to determine the effects of KKT on [3H]TCP binding.

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