Effects of Kamikihito, a Traditional Chinese Medicine, on Neurotransmitter Receptor Binding in the Aged Rat Brain Determined by in Vitro Autoradiography: Changes in Dopamine D₁ and Serotonin 5-HT₂A Receptor Binding

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Using in vitro autoradiography, we investigated the effects of Kamikihito (KKT), a traditional Chinese medicine, on specific [³H](SCH23390 binding to dopamine D₁ receptors and [³H]ketanserin binding to serotonin 5-HT₂A receptors in the rat brain. Specific binding of both compounds was affected by aging. Long-term administration of KKT resulted in decreases in [³H](SCH23390 binding to the cortex and hippocampus in aged rats, and in decreases in [³H]ketanserin binding to the caudate/putamen in young rats. These results suggest that the changes in dopamine D₁ and serotonin 5-HT₂A receptor binding may be involved in the central effects of KKT.

Keywords Kamikihito; [³H](SCH23390 binding; [³H]ketanserin binding; aged rat; autoradiography

Kamikihito (KKT) consists of Astragalus, Ginseng, Atractylodes, Hoelen, Polyaiga, Juabe, Longan, Zizyphus, Angelica, Licorice, Ginger, Saussurea, Bupleurum and Gardenia. This traditional Chinese medicine has been used for the treatment of anemia, insomnia, anxiety, and neurosis. In addition to exerting an anxiolytic effect, KKT has been reported to improve the cognitive ability of alcoholic patients. Further, it has been shown that KKT improves learning performance in the senescent accelerated mouse. Using in vitro quantitative autoradiography, we have recently demonstrated that long-term administration of KKT modulates the binding of [³H]-quinuclidinyl benzilate (QNB) and [³H]N-(1-[2-thienyl]-cyclohexyl)-3,4-piperidine (TCP) to rat brain slices. Although our previous findings suggest a possible involvement of both the cholinergic and glutamatergic neuronal systems in the effects of this agent, little is known about its effects on other neuronal systems. In this study, using in vitro quantitative autoradiography, we investigated the effects of long-term administration of KKT on specific [³H](SCH23390 binding to dopamine D₁ receptors and [³H]ketanserin binding to serotonin 5-HT₂A receptors in the brains of young and aged rats.

MATERIALS AND METHODS

The aged animals used were male Fischer rats (99 weeks old before the experiment; Charles River Japan, Inc.). The young control rats were 6 weeks old before the experiment. All animals were kept in a temperature- and light-controlled room (23°C; 12-h light cycle starting at 9:00 a.m.). Animals were given a regular diet or one containing KKT (8%, Kanebo Co., Ltd.) for 15 weeks before they were killed. After 15 weeks of the experimental regimen, they (rats aged 114 and 21 weeks) were sacrificed for autoradiographic analysis. The calculated daily doses of KKT in the young and aged rats were 1.25 and 1.36 g/rat, respectively. The body weights of the aged rats given the regular and KKT-containing diets after the 15-week period of drug administration were 484 ± 4 (n = 5) and 461 ± 5 g (n = 5), respectively, while those of young rats given the regular and KKT-containing diets were 287 ± 17 (n = 5) and 278 ± 15 g (n = 5), respectively. The rats were sacrificed by decapitation and their brains were rapidly frozen at −100°C. Twenty-μm cryostat sections were prepared for the binding assay. The autoradiography of [³H](SCH23390 (2 nM; specific activity, 87.0 Ci/mmol, NEN Research Products) and [³H]ketanserin (2 nM; specific activity, 64.1 Ci/mmol, NEN Research Products) binding was carried out as described previously. Non-specific binding of [³H](SCH23390 and [³H]ketanserin was defined as the binding in the presence of 100 μM SCH23390 (Schering), and 1 μM methysegride (Sandoz), respectively. The specific binding of [³H](SCH23390 and [³H]ketanserin represented approximately 85% and 50% of the total binding, respectively. Results were expressed as the means ± S.E. (n = 5). The significance of differences was assessed by a two-tailed Student's t-test.

RESULTS

As shown in Fig. 1, the specific binding of [³H](SCH23390 (2 nM) to the cortex in aged rats was significantly higher than that in young rats (young rats: 0.086 ± 0.003, aged rats; 0.095 ± 0.002 pmol/mg protein), while binding to the caudate/putamen (young rats: 0.356 ± 0.002, aged rats; 0.339 ± 0.003 pmol/mg protein) and N. accumbens (young rats; 0.339 ± 0.003, aged rats; 0.303 ± 0.008) in aged rats was significantly lower than that in young rats. The [³H](SCH23390 binding to the hippocampus and substantia nigra in aged rats did not differ from those in young rats. Long-term administration

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Fig. 1. \([^{3}H]\)SCH23390 (2 nm) Binding to Various Brain Regions in Young and Aged Rats Given Regular or KKT (8%)-Containing Diets for 15 Weeks

- ■, young rats given regular diet; □, aged rats given regular diet; ◇, young rats given KKT-containing diet; □, aged rats given KKT-containing diet. Each value represents the mean ± S.E. \((n=5)\). *a* indicates a significant difference at \(p<0.05\).

Fig. 2. \([^{3}H]\)Ketanserine (2 nm) Binding to Various Brain Regions in Young and Aged Rats Given Regular or KKT (8%)-Containing Diets for 15 Weeks

- ■, young rats given regular diet; □, aged rats given regular diet; ◇, young rats given KKT-containing diet; □, aged rats given KKT-containing diet. Each value represents the mean ± S.E. \((n=5)\). *a* indicates a significant difference at \(p<0.05\).

of KKT in young rats had no effect on \([^{3}H]\)SCH23390 binding to any brain regions examined. In contrast, \([^{3}H]\)SCH23390 binding to the cortex and hippocampus in aged rats was significantly decreased by the long-term administration of KKT (the specific binding to the cortex and hippocampus in the control aged rats was \(0.095 \pm 0.002\) and \(0.076 \pm 0.004 \text{ pmol/mg protein} \), respectively, while in KKT-treated aged rats, it was \(0.077 \pm 0.008\) and \(0.061 \pm 0.003 \text{ pmol/mg protein} \), respectively.).

As shown in Fig 2, specific \([^{3}H]\)ketanserine (2 nm) binding to the cortex (young rats; \(0.112 \pm 0.007\), aged rats; \(0.089 \pm 0.009 \text{ pmol/mg protein} \)) and N. accumbens (young rats; \(0.093 \pm 0.006\), aged rats; \(0.067 \pm 0.009 \text{ pmol/mg protein} \)) was significantly decreased in aged rats compared with young rats. This binding to the caudate/putamen in young rats was significantly decreased by the long-term administration of KKT (control young rats; \(0.039 \pm 0.001\), KKT-treated young rats; \(0.030 \pm 0.004 \text{ pmol/mg protein} \)). Long-term administration of KKT in aged rats had no effect on \([^{3}H]\)ketanserine binding to any brain regions examined.

**DISCUSSION**

In previous studies,\(^6\)\(^-\)\(^8\) we found that in aged rats, not only was learning and memory impaired, but emotional
behavior was also altered. In the present study, we observed a significant increase in the specific binding of \(^{3}H\)SCH23390 to the cortex of aged rats compared with that in young rats. In the elderly, psychotic symptoms, including delusions and hallucinations, are frequently observed in addition to memory impairment, these psychotic symptoms being sensitive to treatment with neuroleptics.\(^9\)

It has been reported that \(^{3}H\)SCH23390 binding to the frontal cortex, determined by positron emission tomography, is decreased in subjects with mood disorders.\(^10\) Therefore, it is possible that the increase in specific \(^{3}H\)SCH23390 binding to the cortex in aged rats may be responsible, at least in part, for the altered emotional behavior in aged rats. Further investigation of DA receptors, including D\(_2\) receptors, in the cortices of aged rats may help to clarify the neurochemical basis of the psychotic symptoms that are exhibited by elderly humans. In contrast to the increase in the specific binding of \(^{3}H\)SCH23390 to the cortex in aged rats, this binding to the caudate/putamen and N. accumbens in the aged rats was significantly decreased, a finding that is in agreement with a previous study.\(^11\) In an earlier study, we demonstrated that locomotor activity was decreased in aged rats compared with young rats.\(^7\) Since DAergic neurons in the striatum and N. accumbens play an important role in the locomotor activity of animals,\(^12\) it is possible that this decrease in the locomotor activity of aged rats may be associated, at least in part, with the reduced binding of the \(^{3}H\)SCH23390 to the caudate/putamen and N. accumbens.

Serotonin plays a role in psychiatric disorders such as depression, schizophrenia and anxiety, as well as in memory and learning.\(^13\) Therefore, we consider that the decrease in specific \(^{3}H\)ketanserin binding to the cortex and N. accumbens in aged rats may be responsible, at least in part, for the impairment of learning and memory and for anxiety-related behavior in aged rats.

Long-term administration of KKT caused a decrease in \(^{3}H\)SCH23390 binding to the cortex and hippocampus in aged rats, while it had no effect in young rats. Since cortical DA receptors are considered to play an important role in moods and psychotic states, as noted above, the reduction of \(^{3}H\)SCH23390 binding to these brain areas in aged rats produced by KKT suggests that this agent may be effective for the treatment of psychotic symptoms in the elderly. Long-term administration of KKT also affected \(^{3}H\)ketanserin binding to the caudate/putamen in young rats. Although the physiological significance of this change in the binding is uncertain, our findings suggest that the changes in dopamine D\(_3\) and serotonin 5-HT\(_2\)A receptor binding may be involved in the central effects of KKT. It is unlikely that the changes in \(^{3}H\)SCH23390 and \(^{3}H\)ketanserin binding in rats administered with KKT are due to the direct binding of a constituent of KKT to the \(^{3}H\)SCH23390 and \(^{3}H\)ketanserin binding sites, since the brain sections were preincubated to remove substances which had previously bound to these sites before incubating the tissues with the radioligands. In order to clarify the role of changes in \(^{3}H\)SCH23390 and \(^{3}H\)ketanserin binding during aging, as well as the effects of KKT on these bindings, Scatchard analyses of these bindings should be carried out.

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