

Effects of Bifemelane Hydrochloride, a Brain Function Improver, on Muscarinic Receptors in the CNS of Senescence-Accelerated Mouse

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ABSTRACT—Senescence-accelerated mouse (SAM-P/8) is known as a murine model of aging and memory dysfunction, compared with control mouse (SAM-R/1). In the hippocampus of 9-month-old SAM-P/8, the B_{\max} of [^3H]QNB binding was decreased compared with that of SAM-R/1 at the same age. Single and repeated administrations of bifemelane to SAM-P/8 induced an increase in the B_{\max} of [^3H]QNB binding in the hippocampus. From these results, bifemelane seems to exert pharmacological effects through possible activation of the cholinergic system in the hippocampus of SAM.

Senescence-accelerated prone mouse (SAM-P/8), a murine model of accelerating aging, shows an earlier onset (about 8 months after birth) and irreversible advancement of senescence manifested by the following signs and lesions: a loss of activity, alopecia and lack of hair glossiness, skin coarseness, periophthalmic lesions, a short life span, and systemic senile amyloidosis (1). SAM-P/8 shows an age-related deterioration of learning ability: a significant avoidance deficit in the passive avoidance task and prolongation of performance time in the multiple-T maze task, compared with control mouse, senescence-accelerated resistant mouse (SAM-R/1) (2). Thus, SAM-P/8 may prove to be a pertinent model for studying the mechanisms related to the memory deficit seen in senile humans.

Recent neurochemical investigations on patients with Alzheimer's disease (AD) and senile dementia of the Alzheimer's type (SDAT) have demonstrated cholinergic dysfunctions in the cerebral cortex and hippocampus (3, 4): a decrease in muscarinic receptor density and marked reductions in the activity

of choline acetyltransferase and/or acetylcholinesterase. In humans, moreover, inhibition of central cholinergic neurotransmission by treatment of scopolamine results in losses of memory and cognition that are similar to those in patients with AD and/or SDAT. In mice, scopolamine also induced the dysfunctions of learning and memory.

Bifemelane hydrochloride, 4-(*o*-benzylphenoxy)-*N*-methyl-butylamine hydrochloride, is a clinically effective nootropic used in Japan for the treatment of the decrease in cognitive and emotional disturbances related to cerebrovascular disease. This drug improves scopolamine-induced memory deficits in rats (5) and enhances ACh release evoked by high potassium (6). These results suggest the possible involvement of the cerebral cholinergic system in the effects of bifemelane hydrochloride. We here examined the effects of single and repeated administration of bifemelane hydrochloride on specific [^3H]QNB binding in the cerebral cortex and hippocampus of SAM-P/8, and we compared the binding parameters between SAM-P/8 and SAM-R/1 at 9 months

when significant differences in the learning and memory functions have clearly appeared.

L-[Benzilic-4,4'-³H]quinuclidinyl benzilate (QNB, 1624.3 GBq/mmol) was purchased from New England Nuclear. Atropine was purchased from Wako Chemicals, and bifemelane hydrochloride was a generous gift from Eisai Co. (Japan).

SAM-R/1 and P/8 were originally a gift from Dr. Toshio Takeda, Professor of the Chest Disease Research Institute (Kyoto University, Japan). SAMs were bred under conventional conditions, housed at $23 \pm 1^\circ\text{C}$, and allowed free access to food and water; the light-dark cycle was set at 12 hours. For single dosing, male and 9-month-old SAMs (3 mice in one group) were intraperitoneally (i.p.) administered bifemelane hydrochloride (0, 10 or 25 mg/kg; 0.1 ml in saline/10 g weight). For repeated administration, same aged SAMs (3 mice in one group) were given bifemelane hydrochloride (0 or 10 mg/kg; 0.1 ml/10 g weight, i.p.) once a day for 10 days. The corresponding controls were dosed with saline (0.1 ml/10 g weight). At two hours after the dosing in the single administration and at 24 hours after the final dosing in the 10-days administration, SAMs were decapitated, their brains were rapidly removed, and the cerebral cortex and hippocampus were dissected from the brains on an ice-cold plastic plate. The cerebral cortex and hippocampus were then homogenated with 10 volumes of 50 mM Tris-HCl buffer (pH 7.4), and these homogenates were centrifuged at $50,000 \times g$ for 20 min. The pellets thus obtained were washed three times with the same buffer. The sedimented membranes of the cerebral cortex and hippocampus of SAM were suspended (2–5 mg/ml) in 50 mM Tris-HCl buffer (pH 7.4) and stored at -80°C .

The reaction mixture (500 μl) contained 50 mM Tris-HCl (pH 7.4), the membranes (final concentration: 50–100 μg protein/ml) and the radioligand [³H]QNB (0.2–0.4 nM), in the presence (in the assay of repeated administration) or absence (in the assay of single administration) of 100 mM NaCl and 25 mM

MgCl₂. After the reaction mixture was incubated at 30°C for 90 min, it was filtered under vacuum through a Whatman GF/C filter, and the filter was washed three times with 1.5 ml of ice-cold buffer. Specific binding was defined as radioactivity bound after subtraction of the nonspecific binding determined in the presence of 1 μM atropine, from the total binding. The saturated radioligand-binding assay was carried out in triplicate and the data were subjected to Scatchard analysis to determine the values of K_D and B_{max} , by a computerized linear least-squares curve-fitting procedure using a one-site model. Statistical differences between the drug-treated and vehicle groups were analyzed using the two-tailed *t*-test. Protein concentration was determined by Lowry's method with bovine serum albumin as a standard.

The body weights of 9-month-old SAM-P/8 (27.1 ± 0.6 g, $n = 9$) were significantly lower than those of the age-matched SAM-R/1 (32.3 ± 0.3 g, $n = 3$). The wet weights of the hippocampus and cerebral cortex, however, were not different between SAM-P/8 and SAM-R/1. In hippocampal membranes of 9-month-old SAM-P/8, the B_{max} of [³H]QNB binding was significantly ($P < 0.05$) decreased versus that of same aged SAM-R/1, without any change in the K_D (Fig. 1, C and D). In comparison, the K_D and B_{max} of [³H]QNB binding to cerebral cortical membranes were not different between SAM-P/8 and SAM-R/1 (Fig. 1, A and B).

In the first assay, [³H]QNB binding was performed in the absence of NaCl and MgCl₂, the condition in which there is stabilization and maximum sensitivity for the antagonist. If bifemelane hydrochloride induces qualitative changes of muscarinic receptors, the K_D value of [³H]QNB binding in the absence of NaCl and MgCl₂ should be changed by the administration of this drug. However, the K_D value of [³H]QNB binding did not change in cerebral cortical and hippocampal membranes of SAM-P/8 administered with this drug (10, 25 mg/kg, i.p.) (Fig. 1, A and C). Only the B_{max} value of [³H]QNB binding was changed by

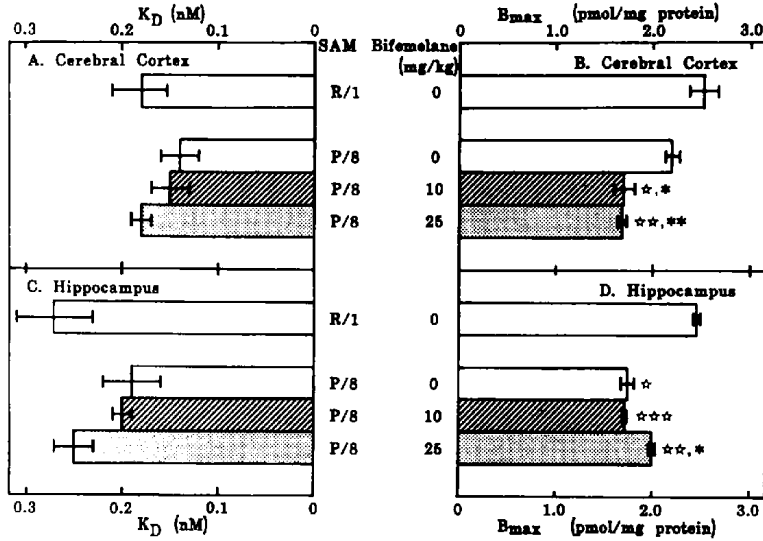


Fig. 1. Effects of single administration of bifemelane hydrochloride on the K_D and B_{max} of $[^3H]QNB$ binding to cerebral cortical and hippocampal membranes. Bifemelane hydrochloride (0, 10 or 25 mg/kg, i.p.) was administered to male 9-month-old SAM-P/8. The animals were sacrificed 2 hours after the dosing. The results are expressed as the means \pm S.E. of three experiments in each group. K_D values in the cerebral cortex and hippocampus are shown in A and C, respectively. B_{max} values in the cerebral cortex and hippocampus are shown in B and D, respectively. Significance of difference: $\star P < 0.05$, $\star\star P < 0.01$, $\star\star\star P < 0.001$ vs. vehicle dosing group in SAM-R/1. $\star P < 0.05$, $\star\star P < 0.01$ vs. vehicle dosing group in SAM-P/8.

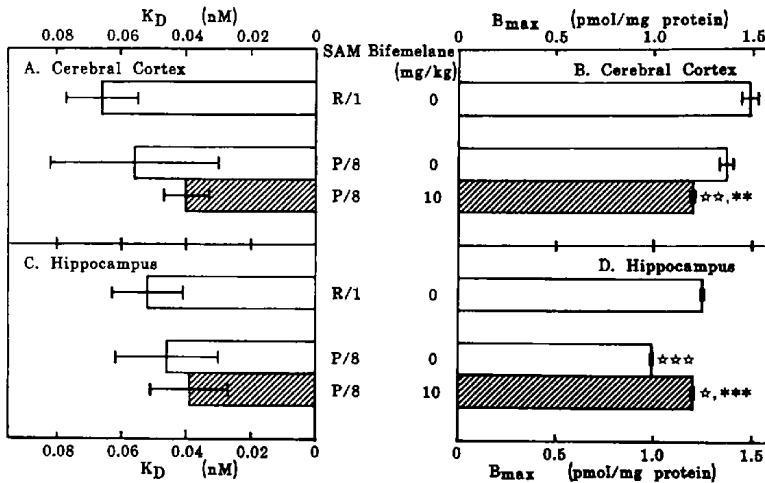


Fig. 2. Effects of repeated administration of bifemelane hydrochloride for 10 days on $[^3H]QNB$ binding in the cerebral cortex and hippocampus. Bifemelane hydrochloride (0 or 10 mg/kg/day, i.p.) was administered to SAM-P/8. The animals were sacrificed 24 hours after the last injection for the assay of $[^3H]QNB$ binding. The results are expressed as the means \pm S.E. of three experiments in each group. The K_D values in the cerebral cortex and hippocampus are shown in A and C, respectively. The B_{max} values in the cerebral cortex and hippocampus are shown in B and D, respectively. Significance of difference: $\star P < 0.05$, $\star\star P < 0.01$, $\star\star\star P < 0.001$ vs. vehicle dosing group in SAM-R/1. $\star\star P < 0.01$, $\star\star\star P < 0.001$ vs. vehicle dosing group in SAM-P/8.

administration of this drug: the value in cerebral cortical membranes was significantly ($P < 0.05$) decreased (Fig. 1, B), and the values in hippocampal membranes was significantly ($P < 0.05$) increased (Fig. 1, D), compared to the values of SAM-P/8 (vehicle administration) and SAM-R/1.

Thus, bifemelane hydrochloride does not affect qualitative changes in the muscarinic receptor. Therefore, we focused on the changes in the B_{\max} values of the binding. In the second assay, [^3H]QNB binding was performed in the presence of 100 mM NaCl and 25 mM MgCl_2 . Long-term administration of bifemelane hydrochloride (10 mg/kg, i.p., for a period of 10 days) to 9-month-old SAM-P/8 increased the B_{\max} value of [^3H]QNB binding in the hippocampus and improved it to the level of SAM-R/1 (Fig. 2, D). This treatment, however, significantly decreased the B_{\max} in the cerebral cortex ($P < 0.01$) (Fig. 2, B).

SAM-P/8 acquires severe forms of degeneration in its appearance that are accompanied by pathological amyloidosis and memory dysfunctions at about 8 months after birth, while such senile signs do not emerge in SAM-R/1 or other normal mice at the same age (1, 2). During the breeding of SAM, we noticed that, such differences in aging syndromes between SAM-R/1 and SAM-P/8 were generally more prominent in males than females, but could not explain why this is so. Therefore, in these studies, we used male animals that were about 9-month-old. Recently, it was reported that dysfunction in the passive avoidance behavior of SAM-P/8 was improved to the activity level of SAM-R/1 by some brain function improvers such as indeloxazine and dihydroergotoxine (7). In addition, SAM-P/8 at 9 months exhibited dysfunction of hippocampal muscarinic receptors (8). It is of interest to note that SAM-P/8 at the same stage lose hippocampal dendritic spines (9).

The B_{\max} of [^3H]QNB binding in the hippocampus was partially improved 2 hours after administration of a high dose (25 mg/kg, i.p.) of bifemelane hydrochloride to SAM-P/8. In addition, repeated administration of this

drug at a low dose (10 mg/kg, i.p., a period for 10 days) caused an increase in the B_{\max} in the hippocampus of SAM-P/8 and recovered it to the level of SAM-R/1, normal aging mice. The binding of [^3H]QNB in vitro, however, was not changed by the addition of bifemelane hydrochloride up to $1\ \mu\text{M}$ (data not shown). From these results, bifemelane might cause an improvement of muscarinic receptors impaired in the hippocampus of SAM-P/8. Recently, it has been reported that bifemelane hydrochloride suppressed the decrease in ACh concentration in the cerebral cortex and hippocampus of ischemic gerbils (10), the decrease in [^3H]QNB binding in the hippocampus of aging rats, and decreases in learning and memory activities of scopolamine-treated rats (11). Furthermore, bifemelane hydrochloride augments the long-term potentiation in guinea pig hippocampal slices (12) through possible activation of protein kinase C (13), but the effect of this drug is inhibited by scopolamine. Protein kinase C is activated by diacylglycerol formed by the stimulation of phospholipase C via muscarinic receptors in the CNS (14). Therefore, this drug seems to exhibit pharmacological effects through possible activation of the cholinergic mechanism in the CNS.

Although the B_{\max} of [^3H]QNB binding in the cerebral cortex of SAM-P/8 was similar to that of SAM-R/1 (normal aging mice), single and repeated administration of bifemelane hydrochloride significantly decreased the B_{\max} ; the reason for this decrease is unclear at present. An interesting result has been reported that bifemelane hydrochloride facilitates ACh release (6) and that the administration of this drug (10, 30 mg/kg, p.o.) decreases the B_{\max} and K_D of [^3H]QNB binding to forebrain membranes in normal rats (15). Therefore, muscarinic receptors in the cerebral cortex of SAM-P/8 were possibly desensitized by administration of bifemelane hydrochloride. In addition, this drug may exert different effects on the cerebral cortex and hippocampus. Further experiments are required to explain these effects.

In conclusion, functional loss of muscarinic receptors seem to occur in the hippocampus of SAM-P/8 at 9 months after birth. Bifemelane hydrochloride improves these dysfunctions by increasing the number of receptors in SAM-P/8. This drug is effective on the dementia induced by dysfunction of the cholinergic system, exerting pharmacological effects through possible activation of muscarinic receptors in the hippocampus.

Acknowledgments

We thank Professor T. Takeda (Kyoto Univ.) for the original gift of SAM and Eisai Co., Ltd. (Japan) for their generous gift of bifemelane hydrochloride. This study was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas, "Brain Aging", of the Ministry of Education, Science and Culture, Japan.

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