Depressive Behavior and Alterations in Receptors for Dopamine and 5-Hydroxytryptamine in the Brain of the Senescence Accelerated Mouse (SAM)-P10

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ABSTRACT—The senescence accelerated mouse (SAM) is known as a murine model of aging. SAM consists of senescence accelerated-prone mouse (SAMP) and senescence accelerated-resistant mouse (SAMR). Previous studies reported that SAMP10 exhibits age-related learning impairments and behavioral depression in a tail suspension test after 7 months. We investigated the changes in emotional behavior in a forced swimming test and in receptors for dopamine and 5-hydroxytryptamine (5-HT) in SAMP10. SAMP10 at 8 months showed an increase of immobility in the test compared with SAMR1. Treatment with desipramine (25 mg/kg, i.p., 3 days) in SAMP10 caused a decrease in immobility. In the cortex from SAMP10, [³H]quinpirole binding to D₂/D₃ dopamine receptors increased significantly compared with control SAMR1. In the hippocampus from SAMP10, [³H]8-hydroxy DPAT binding to 5-HT_{1A} receptor increased. In midbrains from SAMP10, bindings of [³H]quinpirole and [³H]8-hydroxy DPAT increased. [³H]SCH23390 binding to D₁/D₅ receptors and [³H]ketanserin binding to 5-HT₂ receptor in brain regions examined in SAMP10 were similar to those in SAMR1. The present findings represent the first neurochemical evidence of an increase of D₂/D₃ and 5-HT_{1A} receptors in SAMP10. SAMP10 may be a useful model of aging associated depressive behavior.

Keywords: Senescence accelerated mouse, Depression, Dopamine, 5-Hydroxytryptamine, Receptor

An animal model for accelerated senescence named the Senescence Accelerated Mouse (SAM) was developed by Prof. Takeda's group in 1970 (for review, see Refs. 1 and 2). The SAM strains were developed from the AKR/J strain. Since selective breeding was based on the findings of senescence, life span and pathologic phenotypes, and in addition, from sufficient generations of sister-brother mating, they can be considered an inbred strain. This strain is a group of related strains including nine strains of accelerated senescence-prone, short-lived mice (SAMP) and three strains of accelerated senescence-resistant, long-lived mice (SAMR). The mean life span in the P series was 9.7 months, 40% shorter than that of the R series (16.3 months). SAMP8 especially shows a dysfunction in learn-

ing and memory (1–3), and previously we reported the changes of neurotransmission by various types of receptors in SAMP8 (for review, see Ref. 4). SAMP10 exhibits age-related brain atrophy and significant learning impairments in an avoidance task after 7 months but not at 2 months (5, 6). Miyamoto (7) reported that SAMP10 exhibited significant behavioral depression compared with control SAMR1 in a tail suspension test, although SAMP8 did not exhibit depression. The forced swimming test has been described by Porsolt et al. (8, 9) as a useful screening test for antidepressants. Non-pharmacological treatments that have proven efficacy in human depression (electroconvulsive shock, REM sleep deprivation etc.), as well as tricyclic and atypical antidepressants, produce a positive effect in the test (10, 11). In this study, we examined whether SAMP10 shows depressive behavior in the forced swimming test.

The biological basis of depressive behavior has been

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dominated by the monoamine hypothesis, focusing on the involvement of dopamine, 5-hydroxytryptamine (5-HT) and noradrenaline. The destruction of brain dopaminergic neuronal systems in rodent and nonhuman primate species leads to behaviors that model a number of human psychopathologic condition (12). The 5-HT and its receptors also affect emotional behavior. Notably, the 5-HT_{1A} receptor appears to be involved in many of the physiological (e.g., mood) and pathophysiological (e.g., anxiety, depression) actions of 5-HT (13, 14). In the present study, we measured the ligand bindings to dopamine and 5-HT receptors in brains from SAMP10 in comparison with those in SAMR1. The obtained findings show that SAMP10 is an animal model showing depressive behavior and changes of D₂ and 5-HT_{1A} receptors.

MATERIALS AND METHODS

Animals

Male SAMR1 and SAMP10 (6- to 8-month-old) mice were fed a commercial diet ad libitum. Male ddY mice (7-week-old) were obtained from Sankyo Lab. Corp. (Sapporo) and kept for 1 week in our laboratory. All animal experiments were carried out in accordance with the NIH Guide for Care and Use of Laboratories Animals and approved by the animal care and use committee in Hokkaido University.

Materials

Imipramine, apomorphine and 5-HT were purchased from Sigma (St. Louis, MO, USA). Desipramine was obtained from Wako Pure Chemicals (Osaka). Sulpiride and SCH23390 (*R*(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine-7-ol) were obtained from Fujisawa Pharm. Corp. (Tokyo) and Shering Corp. Japan (Tokyo), respectively. [³H]Quinpirole (1369 GBq/mmol), [³H]SCH23390 (2601 GBq/mmol), [³H]8-hydroxy DPAT (4621 GBq/mmol) and [³H]ketanserin (2456 GBq/mmol) were obtained from NEMTM Life Sci. Products (Boston, MA, USA).

Measurement of immobility in the forced swimming test

The procedures were as described by Porsolt et al. (8) with minor modifications. Briefly, male ddY mice (8-week-old), SAMR1 or SAMP10 (6- to 7-month-old) were forced to swim in a cylinder (height, 20 cm; diameter, 8 cm) with water (25°C, depth, 10 cm), from which they could not escape. The trial lasted 8 min and the total duration of immobility was measured at 1-min intervals. In all instances, the immobility was measured between 2:00 and 5:00 p.m.

Measurement of ligand binding to receptors

Male SAMR1 and SAMP10 (7- to 8-month-old) were killed by decapitation and the brain was then rapidly removed. The regions of interest were dissected out on an ice-cold plate then and quickly frozen and stored in a deep freezer at -80°C. The sections were homogenized in 10 vol. of ice-cold buffer A (50 mM Tris-HCl, 5 mM KCl, 2 mM MgCl₂, 2 mM CaCl₂) containing protease inhibitors (0.2 mM benzamidine and 0.2 mM phenylmethanesulfonyl fluoride) using a glass-Teflon homogenizer (20 strokes). The homogenate was immediately centrifuged at 1,000 × *g* for 5 min and the supernatant was centrifuged at 40,000 × *g* for 20 min at 4°C. The pellet obtained was washed by resuspension in fresh buffer A and centrifugation. The protein concentration was determined by means of a Bio-Rad assay kit using bovine serum albumin as the standard. For binding experiments, membrane fractions (200 μg protein/tube in cortex, 80 μg/tube in striatum, 50 μg/tube in hippocampus, 200 μg/tube in midbrain) were used, and the total volume of the assay mixture was 200 μl. [³H]Quinpirole binding was carried out as previously reported (15–17) with minor modifications. The assay mixture was incubated for 2 h at 25°C, and non-specific binding was determined using 20 μM sulpiride. [³H]SCH23390 binding was carried out as previously reported (18, 19) with minor modifications. The assay mixture was incubated for 1 h at 25°C, and non-specific binding was determined using 20 μM cold SCH23390. [³H]8-Hydroxy-DPAT binding was carried out as previously reported (20, 21) with minor modifications. The assay mixture was incubated for 45 min at 25°C, and non-specific binding was determined using 20 μM 5-HT. [³H]Ketanserin binding was carried out as previously reported (22–24) with minor modifications. The assay mixture was incubated for 40 min at 25°C, and non-specific binding was determined using 10 μM cold ketanserin.

Statistical significance

Independent data are expressed as the mean ± S.E.M. Simple comparisons between two groups were made using Student's and the paired *t*-test. In vivo data were analyzed using two-way ANOVA with time as the repeated measurement and paired *t*-test. *P* < 0.05 was considered to be significant.

RESULTS

Decrease of immobility by apomorphine and imipramine in ddY mice

First, we confirmed that the forced swimming test under our conditions is suitable for measuring behavioral changes. The ddY mice when placed in the cylinders were initially highly active, vigorously swimming around. After

Fig. 1. Effects of apomorphine and imipramine in the forced swimming test in ddY mice. A: Male ddY mice (8-week-old) were treated with saline ($n=6$) or imipramine (30 mg/kg, i.p., $n=8$) at 10:00 a.m. for 3 days. The forced swimming test was started from 2:00 p.m., 4 h after the injection, on the third day. B: Male ddY mice (8-week-old) were treated with saline ($n=6$) or apomorphine (1 mg/kg, s.c., $n=9$) at 30 min before the forced swimming test. Floating time for each 1-min interval is given as the mean $\pm$ S.E.M. ^aSignificant difference ($P<0.01$) versus control mice.

1–2 min their activity began to decrease and the duration of immobility (floating time) increased (Fig. 1). After 3–4 min the duration of immobility reached a plateau where the mice remained immobile for over 90% of the time. Repeated administration of imipramine (30 mg/kg, 3 days), a tricyclic antidepressant, caused a significant decrease in immobility; the treated mice apparent attempt to escape persisted for 2 min in the test conditions, in contrast with controls (Fig. 1A). Repeated administration of desipramine (25 mg/kg, 3 days) also caused a decrease in immobility in a similar manner (data not shown). A single injection of the same concentration of imipramine (or desipramine) 24 h before the test had no effect (data not shown). These findings are consistent with those of previous reports (9, 11). Next, we investigated the effect of apomorphine, an agonist for dopamine receptors (Fig. 1B). A single injection of apomorphine (1 mg/kg, i.p.) 30 min before the test caused a remarkable decrease in immobility; the duration of immobility was reduced for 7 min in the treated mice. At 8 min, the behavioral pattern was the same as the treated and control mice.

Increase of immobility in SAMP10

In SAMP10, age-related impairment of learning/memory was observed (5, 6). Miyamoto (7) reported that SAMP10 at 8-month-old exhibited significant behavioral depression in the tail suspension test and also mentioned behavioral depression in the forced swimming test (data not shown). In the present study, we compared the behavioral change of SAMP10 (6- to 7-month-old) with that of SAMR1 (6- to

7-month-old) in the forced swimming test (Fig. 2). SAMR1 were initially highly active and then after 1–2 min, began to decrease their activity level as the duration of immobility increased gradually (Fig. 2A). After 3 min, the duration of immobility in SAMR1 reached a plateau where the mice remained immobile for over 60% of the time. In SAMP10, the duration of immobility was significantly longer than that in SAMR1 at 2, 3 and 5 min. The total floating time up to 3 min from the start of the test on the first day was 102 ± 8 s in SAMP10, which was significantly longer than the value (61 ± 10 s) in the control SAMR1. Next, we conducted the test on 3 consecutive days, with one trial each day. On the third day (trial), the total floating time up to 3 min was similar in both SAMP10 and SAMR1: 139 ± 6 and 129 ± 4 s, respectively. Repeated administration (25 mg/kg, 3 days) of desipramine in SAMP10 caused a significant decrease in immobility (Fig. 2B). Repeated administration (30 mg/kg, 3 days) of imipramine in SAMP10 also caused a decrease in immobility (data not shown). These findings show that the depressive behavior in SAMP10, compared with the control SAMR1, was observed in the first day (trial) and that SAMP10 is sensitive to the antidepressants in the forced swimming test.

Increase of [³H]quinpirole binding to D₂ receptors in SAM brain membranes

Next, we investigated the changes of receptors for dopamine by binding assay used radioactive ligands in SAMP10 and SAMR1. Table 1 shows [³H]quinpirole binding in membrane fractions from the indicated brain regions in

Fig. 2. Increase of floating time in the forced swimming test in SAMP10. A: Floating time in the forced swimming test was measured in male SAMR1 (6- to 7-month-old, $n=7$) and SAMP10 (6- to 7-month-old, $n=7$). Floating time for each 1-min interval is given as the mean $\pm$ S.E.M. ^aSignificant difference ($P<0.01$) versus SAMR1. B: SAMP10 (7-month-old) were treated with saline ($n=9$) or desipramine (25 mg/kg, i.p., $n=7$) at 11:00 a.m. for 3 days. The forced swimming test was started from 3:00 p.m., 4 h after the injection, on the third day. ^aSignificant difference ($P<0.01$) versus control (saline-treated) SAMP10.

Table 1. [3 H]Quinpirole and [3 H]SCH23390 binding in SAM brain membranes

Region	Concentration	Ligand binding (fmol/mg protein)	
		SAMR1	SAMP10
[³H]Quinpirole			
Cortex	2.0 nM	3.0 ± 0.4	4.3 ± 0.3 ^a
Striatum	3.6 nM	7.0 ± 0.5	6.8 ± 0.5
Midbrain	3.0 nM	6.0 ± 0.3	9.1 ± 1.0 ^a
Cerebellum	3.1 nM	4.4 ± 0.9	3.8 ± 0.6
[³H]SCH23390			
Cortex	1.8 nM	226 ± 13	246 ± 8
Hippocampus	1.3 nM	95.5 ± 5.4	107 ± 8
Midbrain	1.5 nM	92.5 ± 12.1	90.8 ± 9.2

Membranes from the indicated brain regions were incubated with the indicated concentrations of [3 H]quinpirole or [3 H]SCH23390. Specific binding was determined as described in Materials and Methods. Data are the mean $\pm$ S.E.M. for SAMR1 (7- to 8-month-old, $n=7$) and SAMP10 (7- to 8-month-old, $n=9$), with each experiment carried out in triplicate. ^aSignificant difference ($P<0.01$) versus SAMR1.

SAMR1 and SAMP10. [3 H]Quinpirole is an agonist with high affinity for the D_2 and D_3 dopamine receptor subtypes. In this experiment, we used 2.0–3.6 nM [3 H]quinpirole, which appeared to be close to the K_d value of previous studies (15–17). Although the value of specific [3 H]quinpirole binding in the cortex from control SAMR1 was close to that obtained in Sprague-Dawley rats using 2 nM [3 H]quinpirole (16), the value in the striatum from SAMR1 was much less than that obtained from the rats. There was no significant change of [3 H]quinpirole binding in the striatum and cerebellum between SAMP10 and SAMR1. In the cortex and midbrain, the values of [3 H]quinpirole binding in SAMP10 were significantly higher than

those in SAMR1.

Scatchard analysis using different concentrations of ligand is useful for determining whether the changes of ligand binding are derived from the change of ligand affinity for receptors or from the change in the number of receptors. However, we could not carry out this assay because of limited brain preparations. Thus, we investigated the [3 H]quinpirole binding at 44.6 nM [3 H]quinpirole, a concentration 10–20-fold higher than the reported K_d values, in midbrain membranes. The binding in SAMP10 increased significantly compared with that in SAMR1; the [3 H]quinpirole binding was 28.3 ± 4.2 and 45.1 ± 4.1 (fmol/mg protein, $n=7-9$) in SAMR1 and SAMP10, respectively. In comparison, the [3 H]quinpirole binding at 11.6 nM in hippocampal membranes was determined. The binding in SAMP10 increased slightly but not significantly compared with that in SAMR1; the binding was 19.7 ± 2.2 and 22.8 ± 2.0 (fmol/mg protein, $n=7-8$) in SAMR1 and SAMP10, respectively. These findings suggest that 1) [3 H]quinpirole binding increased in the cortex and midbrain of SAMP10 compared with that of SAMR1, and 2) the increase in midbrain membranes of SAMP10 was at least partially due to the increase in receptor numbers.

[3 H]SCH23390 binding to D_1/D_5 receptors in SAM brain membranes

Next we investigated the binding of [3 H]SCH23390, which binds D_1 -like (D_1 and D_5) receptors as an antagonist (25) in the brain (Table 1). In this experiment, we used 1.38–1.85 nM [3 H]SCH23390, which appeared to be close to the K_d value in the references (18, 19). There was no significant change of [3 H]SCH23390 binding in the brain regions examined between SAMR1 and SAMP10.

Table 2. [^3H]8-Hydroxy DPAT and [^3H]ketanserin binding in SAM brain membranes

Region	Concentration	Ligand binding (fmol/mg protein)	
		SAMR1	SAMP10
[³H]8-Hydroxy DPAT			
Hippocampus	3.5 nM	157 ± 10	210 ± 9 ^a
Midbrain	4.4 nM	30.4 ± 2.6	37.0 ± 2.3 ^a
[³H]Ketanserin			
Cortex	2.0 nM	138 ± 4	138 ± 4
Striatum	2.0 nM	188 ± 12	182 ± 11
Hippocampus	2.0 nM	37.2 ± 2.3	41.1 ± 0.9
Midbrain	2.0 nM	78.7 ± 7.0	65.7 ± 5.3

Membranes from the indicated brain regions were incubated with the indicated concentrations of [^3H]8-hydroxy DPAT or [^3H]ketanserin. Specific binding was determined as described in Materials and Methods. Data are the mean $\pm$ S.E.M. for SAMR1 (7- to 8-month-old, $n=7$) and SAMP10 (7- to 8-month-old, $n=9$) in the experiment on [^3H]8-hydroxy DPAT and for SAMR1 (9-month-old, $n=4$) and SAMP10 (9-month-old, $n=5$) in the experiment on [^3H]ketanserin, with each experiment carried out in triplicate. ^aSignificant difference ($P<0.01$) versus SAMR1.

Increase of [^3H]8-hydroxy DPAT binding to 5-HT_{1A} receptors in SAM brain membranes

The 5-HT receptor can be classified into at least seven major classes labeled 5-HT₁ through 5-HT₇ with further subdivisions in some of the classes such as the 5-HT₁ class into A–F, and the 5-HT_{1A} receptor which appears to be involved in emotional disorders induced by 5-HT (13). We investigated the binding of [^3H]8-hydroxy DPAT, which binds 5-HT_{1A} receptor as an agonist in the brain (Table 2). In this experiment, we used 3.5–4.4 nM [^3H]8-hydroxy DPAT, which appeared to be close to the K_d value from previous studies (20, 21). The value of specific [^3H]8-hydroxy DPAT binding in the hippocampus from SAMR1 was similar to that obtained in Fisher 344 rats using 2 nM [^3H]8-hydroxy DPAT (21). Significant increases in the binding in the hippocampus and midbrain were observed in SAMP10 compared with SAMR1.

[^3H]Ketanserin binding to 5-HT₂ receptors in SAM brain membranes

We investigated the binding of [^3H]ketanserin, which binds 5-HT₂ receptor as an antagonist in the brain (Table 2). In this experiment, we used 2.0 nM [^3H]ketanserin, which appeared to be close to the K_d value from previous studies (22–24). There was no significant change of [^3H]ketanserin binding between SAMR1 and SAMP10 in the brain regions examined.

DISCUSSION

Depressive behavior in SAMP10

The forced swimming test provides insight into both the mechanisms of the clinical effects of drugs and the regulation of behavioral changes induced by receptor stimulation with neurotransmitters (8–11). In the present study, we confirmed that treatment with imipramine or apomorphine had pharmacological effects on ddY mice in the forced swimming test (Fig. 1). Shimada et al. (5, 6) reported that SAMP10 at 7 months, but not at 4 months, showed prominent atrophy in the cortex and learning impairments in an avoidance task. Thus, we conducted a forced swimming test of SAMP10 at 6–7 months and found depressive behavior compared with control SAMR1 (Fig. 2A). This finding is consistent with a previous report showing emotional change in SAMP10 at 8 months as measured by the tail suspension test (3). Since desipramine, an antidepressant, caused a decrease in immobility in SAMP10 (Fig. 2B), SAMP10 appears to be a model of antidepressant-sensitive depressive behavior. In this experiment, we could not detect any significant change of sensitivity to desipramine in SAMR1 and SAMP10 at 8 months. It remains to be determined whether the sensitivity to antidepressants in SAMP10 is dependent on age or not in the future.

Involvement of dopamine and 5-HT receptors in depression

An involvement of dopamine in depression is proposed. Parkinson's disease, which is established as dopaminergic dysfunction, is associated with a high incidence of depression (26). Concentrations of dopamine metabolites, homovanillic acid (27–29) and dihydroxyphenylacetic acid (30), were lower in the cerebrospinal fluid of depressed patients compared with controls, implying a reduced central dopamine turnover. Also, in SAMP10, dopamine content decreased in the cortex, hippocampus and midbrain, and homovanillic acid content decreased in the hippocampus (M. Miyamoto, personal communication). Therefore, we investigated the ligand bindings to dopamine receptors in SAMP10.

Quinpirole is an agonist for D₂ and D₃ receptors, and [^3H]quinpirole binding was increased in the cortex and midbrain membranes of SAMP10 compared with those of SAMR1 (Table 1). Sokoloff et al. (31) found that the D₃ subtype differed greatly from other subtypes; the mRNA encoding the dopamine D₃ receptor exhibited a unique distribution in rat brain, being most abundant in islands of Calleja, olfactory tubercle, with very little mRNA found in the striatum. In the rat cortex and cerebellum, no mRNA of D₃ receptors was detected. Thus, the [^3H]quinpirole binding in SAM brain appeared to be due to D₂ receptors. Since an increase in the midbrain of SAMP10 was observed

when we used 40 nM [^3H]quinpirole, the change appeared to be due to an increase in receptor numbers. [^3H]SCH23390 binding to D_1/D_5 receptors was not changed in the regions examined (Table 1). These findings suggest that 1) the number of D_2 receptors increased in the cortex and midbrain, but not in the striatum and cerebellum, and 2) the number of D_1/D_5 receptors was not affected in the cortex, hippocampus and midbrain of SAMP10 compared with those of SAMR1.

Previously, it was reported that lower levels of a 5-HT metabolite, 5-hydroxyindoleacetic acid, were found in the cerebral spinal fluid of depressed patients (32) and the brain of suicide victims (33), compared with controls. In the hippocampus and midbrain of SAMP10, 5-hydroxyindoleacetic acid content decreased significantly compared with that in SAMR1 (M. Miyamoto, personal communication). In both brain regions of SAMP10, [^3H]8-hydroxy DPAT binding to 5-HT $_{1A}$ receptors increased significantly compared with that in SAMR1 (Table 2). However, [^3H]ketanserin binding to 5-HT $_2$ receptors in the cortex, hippocampus, striatum and midbrain was not changed. The selective activation of postsynaptic 5-HT $_{1A}$ receptors induces an antidepressant response (34), and the hypothermic response to 5-HT $_{1A}$ agonists is blunted in patients with depressive disorders (35). The 5-HT $_{1A}$ receptors are increased in the dorsal raphe nucleus from suicide victims with major depression compared with normal control subjects (36). These findings suggest the involvement of 5-HT $_{1A}$ receptors in depression. It was reported that there were no differences in the number and affinity of D_1 and D_2 receptors in the caudate, putamen and nucleus accumbens between suicide victims free of antidepressants and controls (29), and that the number of 5-HT $_2$ receptors was increased in the cortex from the post-mortem brains of depressed patients and suicide victims (37, 38). Thus, the findings concerning D_2 and 5-HT $_2$ receptors in depressed humans are not consistent with those in SAMP10. The reason for this is not clear, but may be related to differences in species or brain preparations.

Problems to be solved

We could not examine the receptor binding of the three ligands in all brain regions in this study, because the receptor binding assay requires a large amount of membrane protein. In addition, the involvement of neurotransmission by α - (39) and β - (10) adrenergic receptors and by adenosine and benzodiazepine receptors (40) in behavioral changes or emotional disorders is proposed. It remains to be determined whether depressive behavior and the changes of neurotransmission induced by dopamine and 5-HT in SAMP10 are dependent on age or not. In addition, the relationship of depressive behavior to learning impairments in SAMP10 should be determined. In conclusion, SAMP10

at 6–8 months showed changes in dopamine and 5-HT receptors and is a useful model with which to study depressive behavior associated with aging.

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