SI-1

DEPRESSION: CELLULAR AND PHYSIOLOGICAL CONSEQUENCES OF STRESS (ANTIDEPRESSANT EFFECT OF SEROTONIN N-ACETYLTRANSFERASE INHIBITOR)

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Abstract

Melatonin is secreted during the hours of darkness and is thought to influence the circadian and seasonal timing of a variety of physiological processes. Serotonin N-acetyltransferase (AA-NAT) which is found to be expressed in pineal gland, retina, and various tissues, catalyses the conversion of serotonin to N-acetylserotonin and is known as the rate-limiting enzyme in the biosynthetic pathway of melatonin. The compounds that modulate the activity of AA-NAT can be used to treat serotonin- and melatonin-related diseases such as insomnia, depression and seasonal affective disorders (SAD). Several assay methods have been developed by which to measure AA-NAT activity. We have also developed a simple, rapid and sensitive AA-NAT assay method that takes advantage of differences in the organic solubilities between acetyl CoA and N-acetyltryptamine. We screened modulators of AA-NAT activity from the water extracts of the medicinal plants. We found MNP1005 which strongly inhibited the activity of AA-NAT (IC_{50} = 2.2 μM). Enzyme inhibitory kinetic studies revealed that MNP1005 exhibited a noncompetitive inhibition toward tryptamine. The antidepressant effect of MNP1005 was investigated on behavioral despair test so called forced swimming test (FST). MNP1005 significantly increased swimming behavior by reducing immobility with treatment of 10 mg/kg when compared to the vehicle-treated control group ($P < 0.05$). This suggests that MNP1005 possesses antidepressant activity. The influence of chronic MNP1005 treatment on the expression of brain-derived neurotrophic factor (BDNF) was examined by *in situ* hybridization and Northern blot. Chronic treatment of MNP1005 blocked the downregulation of BDNF mRNA in the frontal cortex and other cortex regions in response to restraint stress.

Key words : Serotonin N-acetyltransferase, Forced swimming test, Brain-derived neurotrophic factor, Antidepressant

INTRODUCTION

The pineal gland is one of the most remarkable neuroendocrine organs in the body. It is a small gland located in the center of the skull between the two cerebral hemispheres. This organ is influenced by light. In mammals pinealocytes are neither sensitive to light nor possess a clock. Instead, the clock is located in a separate hypothalamic structure, the suprachiasmatic nucleus (SCN) (1, 2). Light stimuli reach the SCN indirectly via the retinohypothalamic pathway. Via a multisynaptic pathway, neurons of the SCN project to the intermediolateral cell column of the spinal cord, which contains cell bodies that innervate the superior cervical ganglion (SCG). Sympathetic postganglionic neurons then ascend to innervate the pineal gland. The pineal gland effects the rest of the body by secreting melatonin (3). In the biosynthesis of melatonin, tryptophan is first converted by tryptophan hydroxylase to 5-hydroxytryptophan, which is decarboxylated to serotonin. Melatonin is synthesized from serotonin by an N-acetylation followed by methylation of the 5'-hydroxy moiety by hydroxyindol-O-methyltransferase (HIOMT). Serotonin N-acetyltransferase (AA-NAT; EC 2.3.1.87) whose activity increases dramatically with the onset of darkness is the rate-limiting enzyme in melatonin synthesis, expressed primarily in the pineal gland and in variable degrees in the various tissues including retinas of some vertebrates and controls the night/day rhythm in melatonin production in the vertebrate pineal gland (4-7). Therefore, a pronounced circadian rhythm in circulating levels of melatonin, which is the highest levels at night, reflects large changes in the activity of pineal AA-NAT (8, 9).

The pineal neurohormone melatonin plays important roles in a broad range of physiological functions, such as the biological regulation of circadian rhythms, modulation of the function of the circadian clock in the suprachiasmatic nucleus (SCN), sleep, mood, and perhaps reproduction, tumor growth, aging, and biological effects on the vertebrate retina (10, 11). Imbalance of the level of melatonin has been implicated in various diseases including delayed sleep phase syndrome, advanced sleep phase syndrome, time zone change (Jet Lag) syndrome, shift work sleep disorder, depression, seasonal affective disorder, and premenstrual syndrome (12-15). Both bright light and ingestion of melatonin may alter the circadian rhythm of melatonin secretion, but the reports on this effect are inconsistent. Many kinds of antidepressant drugs are also known to modulate the secretion of neurotransmitters such as serotonin and melatonin (16). Therefore, the components that modulate AA-NAT activity can be developed into potential candidates of therapeutic drugs for the treatment of affective disorders.

Brain-derived neurotrophic factor (BDNF) is reported to play an important role in the survival of mature neurons as well as damaged neurons in the central nervous system (17, 18). BDNF mRNA has

the widest distribution of the neurotrophins in the CNS, and is abundantly expressed in the neocortex and hippocampus (19-20). The expression of BDNF in hippocampus is dramatically down-regulated in response to acute, as well as repeated immobilization stress (21). Decreased expression of BDNF could contribute to the atrophy and death of stress-sensitive CA3 pyramidal neurons in hippocampus, and could play a role in stress related psychiatric illnesses, including depression and posttraumatic stress disorder (22). The mechanism of antidepressant action, at the cellular level, is not clearly understood. It has been reported that chronic antidepressant treatment blocks the down-regulation of BDNF mRNA levels in the hippocampus in response to the restraint stress. Malberg et al. (2000) reported that increased cell proliferation and increased neuronal number may be a mechanism by which antidepressant treatment overcomes the stress-induced atrophy and loss of hippocampal neurons and may contribute to the therapeutic actions of antidepressant treatment. Moreover, in case of the animal model of depression using forced swimming test (FST), a reduction in escape-oriented behavior, induced by repeated exposure to a container of water from which animal cannot escape, is prevented by administration of antidepressant (24).

In this study, we have examined behavioral changes in animals administered with MNP1005 using the FST. Furthermore, we have also examined that the MNP1005 treatment may block the down-regulation of BDNF in response to restraint stress. The brain regions chosen for study were the frontal cortex, other cortical area and hippocampus, which have been implicated in the pathophysiology and treatment of depression and stress (25-27). Here we report that MNP1005 has a potential activity as an antidepressant in view of that it increased swimming behavior in forced swimming test and maintained the level of BDNF mRNA after restraint stress.

MATERIALS AND METHODS

Materials

[^{3}H]Acetyl CoA was obtained from Amersham Pharmacia Biotech. and Econofluor was from NEN Life Science Products (Boston, MA, USA). Acetyl CoA and tryptamine were purchased from Sigma (St. Louis, MO, USA).

Animals

Adult male Sprague-Dawley rats (180 - 200 g) were obtained from Hyochang Science (Seoul, Korea). The rats lived under a 12 h light/12 h dark regime (LD12:12 with lights off at 19:00 o'clock) for at least 1 week prior to the day of the experiment. For drug treatments, groups of rats were administered fluoxetine (5 mg/kg), MNP1005 (20 mg/kg), or vehicle (0.9% saline) once a day for 21 days via intraperitoneal injection. The rats were subjected to restrain stress (45 min) at 18 hr after the

last drug treatments and then sacrificed immediately. Brains were removed and immediately frozen for *in situ* hybridization, or sections of frontal cortex, other cortical area or hippocampus were dissected for RNA extraction and Northern blot analysis, as described below. All experimental procedures with animals were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Pineal Gland Culture

For *in vitro* organ culture, rats were sacrificed and the pineal glands removed. *In vitro* organ culture of rat pineal glands was carried out by a method described previously (28) with some modifications. Pineal glands were placed directly into ice-cold Dulbecco's modified Eagle's medium (DMEM, GIBCO, Grand Island, NY) containing 10% bovine calf serum and 1% penicillin/ streptomycin. After removal of extraneous tissues, the pineal glands were placed on nylon mesh that rested on DMEM medium. The glands were incubated at 37℃ in a humidified atmosphere of 5% CO_2. They were stimulated with isoproterenol in the absence or presence of propranolol and further incubated for 4 hr.

AA-NAT enzyme assay

The activity of AA-NAT was measured as we previously described (29). Briefly, the rats were sacrificed by decapitation at midnight (00:00 h) under safelight. The pineal glands were removed, frozen in liquid nitrogen, and stored at –70℃ deep freezer until assayed for AA-NAT activity. Pineal glands were individually disrupted by ultrasound in 200 μl ice-cold phosphate buffer (50 mM, pH 6.8). Debris was removed by centrifugation (15,000 g, 5 min, 4℃) and the supernatant was transferred to a new tube. An aliquot of 13 μl of supernatant was incubated in the presence of 5 μl tryptamine-HCl (10 mM), 1 μl acetyl CoA (0.5 mM), and 1 μl [^{3}H]acetyl CoA (3.6 Ci/mmol, 250 μCi/ml). The reaction mixture was then incubated at 37℃ for 30 min and the reaction stopped by dilution with 180 μl ice-cold phosphate buffer (50 mM, pH 6.8). Econofluor (1,2,4-trimethylbenzene (> 99%), 2,5-diphenyloxazole (0.7%, w/v), 1,4-bis(2-methylstyryl)benzene (0.05%, w/v)) was rapidly added to the reaction mixture, and the amount of radiolabeled acetyltryptamine was determined in a liquid scintillation counter.

AA-NAT enzyme kinetics

The apparent K_m value for tryptamine was determined under steady-state condition in the presence of fixed concentration of acetyl-CoA (2mM) and varied concentrations (0.05 - 1 mM) of tryptamine. The assay was performed the same as the standard conditions. The double-reciprocal plot (1/[S] vs 1/V) was used to calculate the K_m constants.

Forced swimming test (FST)

Rats were placed in a cylindrical glass tank (40 cm tall x 18 cm in diameter) of 25℃ water filled to a depth of 20 cm for a 15-min pretest. Injections (saline, MNP1005) were given at 23.5, 5 and 1 hr before a 5-min test swim (Fig. 1). The test swimming session was videotaped for behavioral analysis. Behavior during the test swimming session was scored using a time-sampling method (30) modified from the method traditionally used in the FST. Every 5 sec, one of three behaviors was recorded. Immobility was scored when ethe animal was making the minimum movements necessary to stay afloat. Climbing was scored when the animal was vigorous thrashing movement with its forepaws, usually directed against the side of the tank. Swimming was scored when the animal actively swam around the tank, making movements greater than those necessary to stay afloat. Behavioral results were shown as the total number of counts for each behavioral category of a maximum of 60.

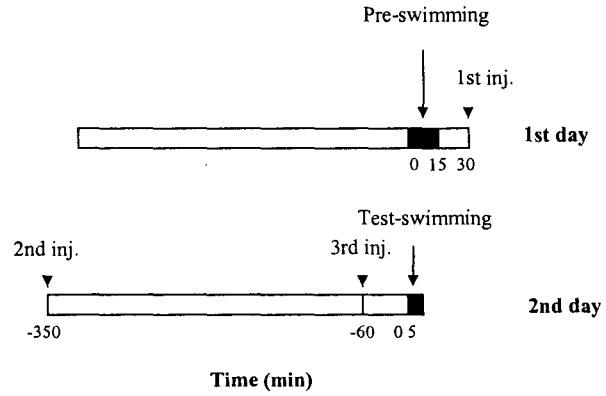

Figure 1. Schematic diagram during the 2-day FST procedure. The swimming periods were separated by 24 hr. During this procedure, drug was administered 3 times at the indicated time intraperitoneally. The 5-min test swimming session was videotaped and analyzed behavioral movement.

Northern blot analysis

Total RNA was extracted from the rat brain tissues by TRI reagent (Molecular research center, INC). Total RNA (15 μg) was resolved by electrophoresis through a 1% agarose gel containing 0.66 M formaldehyde and transferred to nylon membranes (ICN, East Hills, NY). The blots were reacted with a rat BDNF cDNA probe labeled with [α-^{32}P]dCTP by the random primer extension method. The hybridization proceeded at 65℃ in a solution containing 10% polyethyleneglycol, 7% sodium dodecyl sulfate (SDS), 10 mM EDTA, 0.25 M NaCl, 0.085 M Na_2HPO_4 (pH 7.2), denatured salmon

sperm DNA (100 μg per ml), and the radiolabeled probe (5 X 10^5 cpm per ml). After hybridization, the blot was washed briefly one time in 1 X SSC (0.3 M NaCl and 0.03 M sodium citrate) containing 0.1% SDS at room temperature, two times in 0.2 X SSC containing 0.1% SDS at 65℃, and one time in 0.1 X SSC at room temperature.

***In situ* hybridization**

Analysis of BDNF mRNA by *in situ* hybridization was carried out as described previously (31). In brief, coronal sections of 14 μm thickness were cut on the cryostat and thaw mounted onto RNase free probe-on (+) slides (Fisher). Tissue sections were fixed in 4% paraformaldehyde, acetylated and dehydrated. Levels of BDNF mRNA were examined by probing with ^{35}S-labeled riboprobes. The sections were hybridized with 2 x 10^6 cpm/section for 18 hr at 55℃ in hybridization buffer (50% formamide, 0.3 M NaCl, 50 mM Tris, pH 8.0, 5 mM EDTA, 1 X Denhardt's solution, 10% Dextran sulphate, 10 mM DTT, and 400 μg/ml tRNA). After hybridization, sections were washed in 2 X SSC containing 10 mM β-mercaptoethanol and 1 mM EDTA at 25℃ and then treated with 20 μg/ml RNase A for 30 min in RNase buffer (500 mM NaCl, and 10 mM Tris, pH 8.0). The sections were then washed for 10 min in 2 X SSC containing 10 mM β-mercaptoethanol and 1 mM EDTA at room temperature and for 45 min in 0.1 X SSC containing 10 mM β-mercaptoethanol and 1 mM EDTA at 60℃. The sections were then rinsed in 0.5 X SSC. The sections were dried, exposed to Nuclear Emersifier NTB2 (KODAK) and subsequently counterstained with 1% neutral red to allow alignment with the autoradiogram. The specificity of the hybridization was confirmed by demonstrating that ^{35}S-labeled sense BDNF riboprobe did not yield any significant hybridization (data not shown).

RESULTS

AA-NAT assay using rat pineal gland extracts

This assay relies on the selective diffusion of radiolabeled acetyltryptamine into a water-immiscible scintillation fluid (Fig. 2). Unlike organic solvent extraction, thin-layer chromatography, or high performance liquid chromatography, the separation of acetyltryptamine from acetyl CoA and tryptamine is not required in this method. In order to measure the AA-NAT activity quantitatively, linearity of the measurements over a range of enzyme concentrations is required. A linear response curve was obtained by taking measurements after 30 min. Moreover, the limit of sensitivity is less than 4 pmol of N-acetyltryptamine formed per sample (Fig. 3A). Figure 3B show the marked enhancement of AA-NAT activity when *in vitro* organ culture of pineal glands were treated with isoproterenol, an adrenergic receptor agonist. The enhanced AA-NAT activity was inhibited by pretreatment with a β-adrenergic receptor antagonist propranolol.

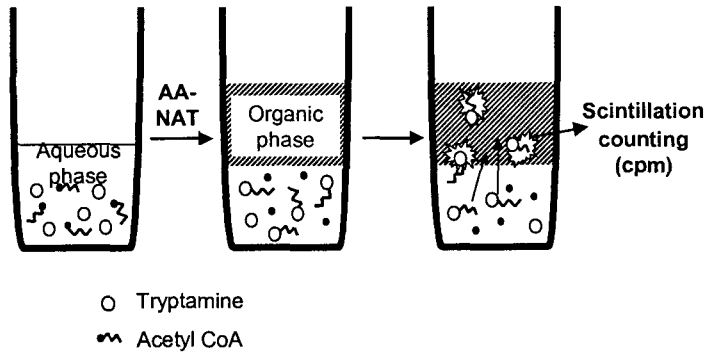

Figure 2. Schematic diagram of liquid biphasic serotonin N-acetyltransferase (AA-NAT) assay. This method utilizes [^{3}H]acetyl CoA and tryptamine as substrates and separates the reaction products in a biphasic liquid system consisting of an aqueous phase and water-immiscible organic scintillation fluid. The radiolabeled acetyltrptamine diffuses into the organic scintillation cocktail, which can be determined by liquid scintillation counting

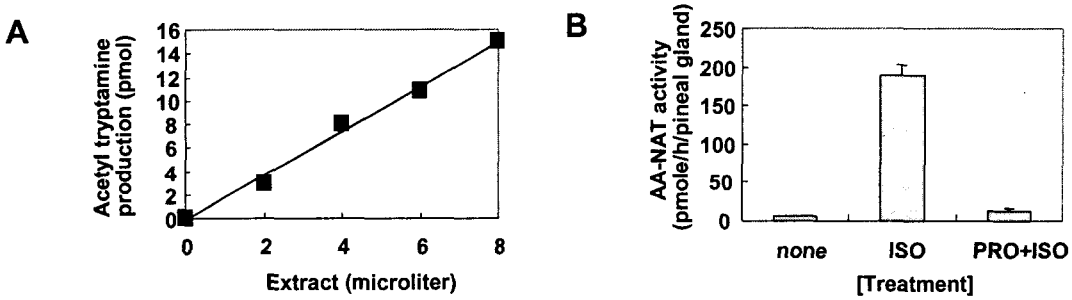

Figure 3. (A): A standard curve of enzyme reaction. The reaction was allowed to proceed for 30 min in the presence of the indicated amounts of pineal gland homogenate, after which the amount of acetyltryptamine produced was measured. Data shown are mean values of duplicate measurements. (B): Increase of AA-NAT activity upon isoproterenol treatment. Cultured pineal glands were incubated for 3 h in DMEM medium, and then treated with 10 μM isoproterenol for 4 h in the absence or presence of 50 μM propranolol. The pineal glands were homogenized and assayed for AA-NAT activity. AA-NAT activity is expressed as pmol/h/pineal gland. Data shown are means ± SEM of three pineal glands.

Effects of MNP1005 on AA-NAT activity

MNP1005 was tested for their effects on AA-NAT activity. To quantify the effect of MNP1005, increasing concentrations of MNP1005 was used. MNP1005 potently inhibited AA-NAT activity with an IC_{50} of 2.2 μM (Fig. 4A). Time-course experiments were also conducted with MNP1005. The addition of 100 μM MNP1005 into the AA-NAT activity assay showed 30% inhibition compared to

the untreated control and the AA-NAT activity fell gradually as the incubation time of MNP1005 passed over 15 min (Fig. 4B). At 15 min, the inhibition of AA-NAT by 100 μM MNP1005 was 45.4%.

The kinetic properties of MNP1005-induced inhibition of AA-NAT activity were further investigated. Figure 5 shows a Lineweaver-Burk analysis of the activity of AA-NAT as a function of various concentrations of the substrate tryptamine ranging from 0.05 to 1 mM. The plot shows a typical non-competitive inhibitory action mechanism of MNP1005 since V_{max}, but not K_m, was affected. The K_m value for tryptamine was 0.463 ± 0.036 mM.

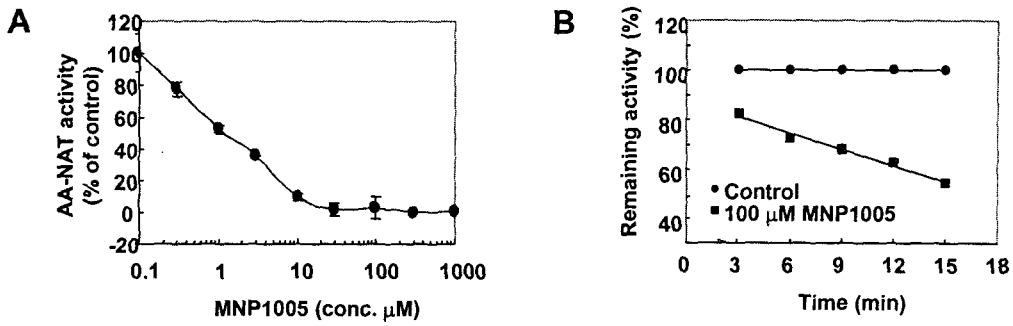

Figure 4. Effect of MNP1005 on AA-NAT activity. (A): MNP1005 produced dose-dependent inhibition in AA-NAT activity with IC_{50} of 2.2 μM. AA-NAT was incubated with indicated concentration of MNP1005 AA-NAT activity was determined by the method described in "Materials and Methods". Each data point represents means ± SEM of triplicate measurements. (B): Time-dependent inhibition of AA-NAT by MNP1005. AA-NAT was incubated with 100 μM MNP1005 for the indicated periods in the assay mixture at 37℃. The AA-NAT activity was measured as described under 'Materials and Methods'. Remaining activities inhibited by MNP1005 are expressed as a percentage of untreated control after each incubation time points. Data are the means ± SEM obtained from triplicate measurements.

Behavior in the Forced Swimming Test

The effect of MNP1005 on behaviors in the FST is shown in Fig. 6. MNP1005 significantly decreased the frequency of immobility counts during the 5-min test session compared to the vehicle-injected control. This was accompanied with a significant increase in swimming behavior but there was no significant difference in climbing behavior after MNP1005 treatment.

The levels of BDNF mRNA by chronic administration of MNP1005

It is reported that chronic antidepressant treatments block stress-induced down-regulation of BDNF mRNA. Studies were under taken to determine if MNP1005 treatments influence the down-

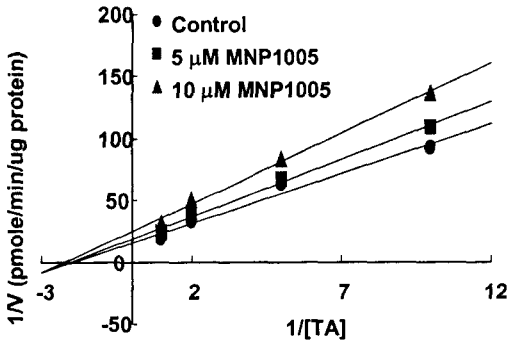

Figure 5. Inhibition kinetics of MNP1005 on AA-NAT. Lineweaver-Burk plots showing non-competitive inhibition toward tryptamine by MNP1005. The AA-NAT activity was measured as described under Materials and Methods. The narious concentrations for tryptamine ranged from 0.05 to 1 mM and 2 mM acetyl- CoA. Data are the means ± SEM obtained from triplicate measurements.

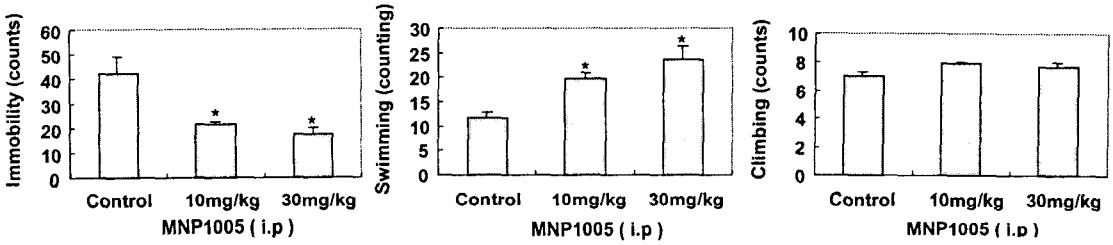

Figure 6. Effect of MNP1005 on immobility, swimming, and climbing behaviors in the FST. Rats were injected with MNP1005 (10 mg/kg and 30 mg/kg) or saline at 23.5, 5 and 1 h prior to the 5-min test swimming period. Mean counts (± SEM) of immobility, swimming, and climbing behaviors are shown when sampled every 5 s during the 5-min test period (n = 8). *$P < 0.05$ compared with control.

regulation of BDNF mRNA by stress as shown by antidepressants. Levels of BDNF mRNA were determined by Northern blot analysis using a riboprobe that recognizes mRNA transcripts (1.8 and 4.4 kb). These mRNA species result from expression of different exons of the BDNF gene and both encode full length of BDNF (32). MNP1005 and fluoxetine was administered chronically for 3 weeks prior to restraint stress (45 min). Levels of BDNF mRNA in the frontal cortex, other cortical areas and hippocampus were decreased by restraint stress compared to non-stress control, confirming the report of Smith et al. (1995) (Fig. 7). Especially, MNP1005 or fluoxetine treatment completely blocked the down-regulation of BDNF mRNA resulting from acute restraint stress in the frontal cortex and other cortical areas (Fig. 7). However, chronic administration of MNP1005 or fluoxetine did not

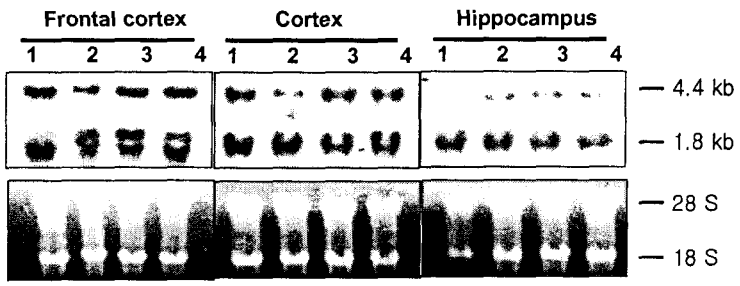

Figure 7. Effect of chronic treatment of MNP1005 on the level of BDNF mRNA. Chronic MNP1005 treatment block the downregulation of BDNF in frontal cortex and cortex by acute restraint stress. Rats were administered saline, fluoxetine (5 mg/kg), or MNP1005 (20 mg/kg) for 21 days or received no stress/saline treatments for the corresponding times. At 18 hours after the last treatments rats were subjected to restraint stress for 45 min, sacrificed immediately, and levels of BDNF mRNA in frontal cortex, other cortical areas, and hippocampus were determined by Northern blot analysis as described in Materials and Methods". 1, no-stress/saline; 2, stress/saline; 3, stress/MNP1005; and 4, stress/ Fluoxetine.

significantly influence levels of BDNF mRNA in the hippocampus (Fig. 7).

To determined whether chronic administration of MNP1005 did not influence the experession levels of BDNF in the subfields of the hippocampus, in situ hybridization analysis with a BDNF cRNA probe used to detect alterations of the levels of BDNF mRNA. In the CA1 and CA3 pyramidal cell layers and dentate gyrus granule cell layer, levels of BDNF mRNA were significantly decreased by the treatment with stress/saline compared to the treatment with no-stress/saline (data not shown). Chronic treatment with MNP1005 increased levels of BDNF mRNA in the subfield of CA3 and dentate gyrus (Fig. 8C). Chronic fluoxetine treatment also increased levels of BDNF mRNA in the CA3 subfield and dentate gyrus (Fig. 8B).

DISCUSSION

We have developed a simple, rapid and sensitive method to measure AA-NAT activity that takes advantage of differences in the organic solubilities between acetyl CoA and N-acetyltryptamine (29).

Pineal gland homogenates were obtained from rats sacrificed at midnight, because peak activity of AA-NAT occurs at 6 hr after the onset of darkness (7). This method exhibited higher sensitivity in assaying AA-NAT activity (< 4 pmole/30 min/sample) as compared to previously reported methods (33, 34). The sensitivity may be further enhanced, if we used [^{3}H]acetyl CoA with high specific radioactivity.

Figure 8. Blockade of stress-induced downregulation of BDNF mRNA in hippocampus by chronic treatmetment with MNP1005. Rats were administered saline, fluoxetine (5 mg/kg), or MNP1005 (20 mg/kg) for 21 days or received no stress/saline treatments for the corresponding times. At 18 hours after the last treatments rats were subjected to restraint stress for 45 min, perfused immediately, and levels of BDNF mRNA in hippocampus were determined by in situ hybridization as described in "Material and Methods". Representative autoradiograms for each of the three treatment conditions are shown. The CA1 pyramidal cell layer (CA1), CA3 pyramidal cell layer (CA3), and dentate gyrus granule cell layer (DG) are indicated. A, saline/stress; B, Fluoxetine/stress; and C, MNP1005/stress.

FST is widely used as an animal model of depression to screen new potent antidepressant drugs in rats or mice (24, 35). This test is sensitive and specific to all major classes of antidepressant drugs including tricyclic antidepressants, selective serotonin reuptake inhibitors, and monoamine oxidase inhibitors (30, 36). The characteristic behavior scored in this test is termed as immobility, swimming, and climbing. Antidepressant drug reduces immobility time, increases swimming, and climbing behaviors, depending on the concentration and the type of antidepressant drug administered. The treatment of MNP1005 significantly reduced the frequency of immobility counts and increased the swimming behavior but not climbing behavior. This indicates that MNP1005 possesses antidepressant activity. There are reports to indicate that immobility, swimming, and climbing behaviors are enhanced by different groups of antidepressant (30). The NE-selective uptake inhibitors enhance the climbing behavior whereas the selective serotonin reuptake inhibitors enhance swimming but not climbing behavior. These results suggest that MNP1005 may elicit specific type of behavior in FST through activating serotonergic systems and thereby acting as an antidepressant agent.

Several studies of stress, depression and action of antidepressant administration suggest that atrophy and death of neurons in the hippocampus, frontal cortex and other brain regions could contribute to the pathophysiology of depression. Several studies also demonstrated that BDNF was a target of antidepressant treatment and was sufficient to produce an antidepressant response. In fact, Siuciak et al. (1996) reported that direct application of BDNF into the midbrain of rats was appeared to have antidepressant effects in behavioral models of depression, including the forced swimming and learned helplessness paradigms. Moreover, chronic administration of different classes of

antidepressants increases the expression of BDNF in the hippocampus and frontal cortex (31, 38). These studies also demonstrate that antidepressant pretreatment blocks the down-regulation of BDNF in response to stress. Our results appear that the administration of chronic MNP1005 or selective serotonin reuptake inhibitor (fluoxetine) induces the blockade of down-regulation of BDNF by restraint stress in the frontal cortex and cortex regions, but not in the hippocampus significantly. However, in the CA3 subfield and dentate gyrus, chronic treatment with MNP1005 increased BDNF mRNA expression compared to treatment with stress/saline using *in situ* hybridization and the MNP1005-induced increase BDNF mRNA was similar to the treatment with fluoxetine. Recent studies have demonstrated that pretreatment with a 5-HT_{2A} antagonist, MDL100,907, reduces the down-regulation of BDNF as shown by antidepressants (39, 40). 5-HT released into the hippocampus during immobility stress could activate 5-HT_{2A} receptors expressed on GABAergic interneurons that inhibit hippocampal neuronal activity by induction of IPSPs in granule cells (40). Because some antidepressant drugs exhibit antagonist properties for 5-HT_{2A} receptors, blockade of BDNF down-regulation could contribute to the action of these drugs. Accordance with these results, we suppose that MNP1005 blocks the stress-induced down-regulation of BDNF mRNA via monoamine-related system involving 5-HT.

Several studies have demonstrated that chronic antidepressant treatment induce up-regulation of the cAMP system at several levels, including increased coupling of Gs and adenylyl cyclase, increased levels of cAMP-dependent protein kinase, and increased expression of cAMP response element binding protein (CREB) (38, 41, 42). In addition, the expression of BDNF in cultured cells is also reported to be up-regulated by activation of the cAMP system (22, 31). CREB can be phosphorylated and activated by cAMP–dependent protein kinase A (PKA) via NE (β-adrenergic receptor) or 5-HT (5-$HT_{4, 6, 7}$), Ca^{2+}-dependent protein kinase via α_1-adrenergic or 5-HT_2 receptors, or CREB kinase which is activated by the MAP kinase pathway (43, 44). These results suggest that up-regulation of the cAMP system and CREB in response to chronic antidepressant treatments influences the expression of specific target genes, one of which is BDNF. Because chronic treatment with MNP1005 also influences the expression of BDNF, we suppose that cAMP signal transduction cascade could be involved in the action of MNP1005 treatment.

This study indicate that MNP1005, which is serotonin N-acetyltransferase inhibitor, may play a role as an antidepressant through the result of behavioral study, which has shown decrease of immobility counts and increase of the swimming behavior by treatment with MNP1005, and blockade of the stress-induced down-regulation of BDNF mRNA in the frontal cortex, other cortical areas, and hippocampus of the rat brain.

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