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Effect of a novel cognition enhancer NS-105 on learned helplessness in rats: Possible involvement of $GABA_B$ receptor up-regulation after repeated treatment

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Abstract

We have previously found that a cognition enhancer [(+)-5-oxo-D-prolinepiperidinamide monohydrate] (NS-105) reversed the inhibition of cyclic AMP formation induced by the $GABA_B$ receptor agonist baclofen. The $GABA_B$ receptor has been implicated in the pathophysiology of depressive illness. The present experiment was designed to evaluate the antidepressant activity of NS-105 in the forced swimming and learned helplessness tests in rats. NS-105 (1–100 mg/kg, p.o.) significantly decreased immobility time in the forced swimming test, an effect similar to that of desipramine. Repeated administration of NS-105 also reversed the failure to escape in the shuttle-box test of rats previously exposed to inescapable footshock. Biochemical data showed that repeated administration of NS-105 increased the number of $GABA_B$ receptors in rat cerebral cortex without affecting the binding properties of β-adrenoceptors and $5\text{-}HT_2$ receptors. In contrast to other antidepressants, NS-105 did not inhibit monoamine uptake in vitro, nor did it change monoamine concentrations in brain tissues or extracellular fluids. These findings suggest that NS-105, which lacks an effect on monoaminergic systems, has potent antidepressant activity, which may involve up-regulation of $GABA_B$ receptors after repeated administration. © 1997 Elsevier Science B.V.

Keywords: Antidepressant; Forced swimming; Learned helplessness; $GABA_B$ receptor

1. Introduction

[(+)-5-oxo-D-prolinepiperidinamide monohydrate] (NS-105) has been developed as a cognition enhancer that reverses learning and memory deficits caused by dysfunction of central cholinergic neurons (Nakagawa et al., 1988, 1990; Ukai et al., 1990). It reverses the decrease in high-affinity choline uptake in rat cerebral cortex induced by electrolytic lesion of the basal forebrain or pentobarbital anesthesia (Ukai et al., 1990).

We have recently found that NS-105 also reverses the memory impairment caused by baclofen, a potent $GABA_B$ receptor agonist (Bowery et al., 1980), in rats. This action was not mimicked by another structurally related cognition enhancer aniracetam (Oka et al., 1995a). In addition, NS-105 inhibited baclofen-induced suppression of cyclic AMP formation in rat cerebral cortex (Oka et al., 1995a,b, 1997). Therefore, it is possible that NS-105 has a functional $GABA_B$ receptor antagonistic action. Recent biochemical evidence has indicated that the $GABA_B$ receptors are involved in the pathophysiology of affective disorders such as depression: chronic administration of a variety of antidepressant drugs or repeated exposure to electroconvulsive shock causes up-regulation of $GABA_B$ receptors in rat cerebral cortex and hippocampus (Lloyd et al., 1985; Gray and Green, 1987; Wojcik and Holopainen, 1992; Pratt and Bowery, 1993). Conversely, down-regulation of $GABA_B$ receptors is observed in the cerebral cortex of olfactory-bulbectomized rats, a possible animal model of depression (Lloyd, 1990).

Therefore, in the present experiment, we tested whether NS-105 possesses antidepressant activity in two representative animal models of depression, namely, forced swimming and learned helplessness in rats.

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2. Materials and methods

2.1. Animals

Male Wistar rats (5–6 weeks old, Charles River Japan, Kanagawa) were used. They were housed in groups of 5–6 in a room kept at 21–25°C with 45–65% humidity and maintained under an alternating 12 h light/dark cycle (lights automatically on at 8.00 a.m.). Food and water were freely available. Experiments were all carried out in accordance with the Guide for the Care and Use of Laboratory Animals of the Japanese Pharmacological Society.

2.2. Chemicals and drugs

The chemicals used in the present experiment were as follows: NS-105 [(+)-5-oxo-D-prolinepiperidinamide monohydrate] was synthesized in our laboratories. Maprotiline hydrochloride, fluoxetine hydrochloride, dopamine hydrobromide and 5-hydroxytryptamine (5-HT) hydrochloride were purchased from Sigma (St. Louis, MO, USA). Noradrenaline bitartrate was obtained from Wako Pure Chemicals (Kyoto, Japan). (±)-Baclofen, nomifensine maleate, isoguvacine hydrochloride, GABA and nipecotic acid were from Funakoshi (Tokyo, Japan). [^{3}H]GABA, [^{3}H]dihydroalprenolol, [^{3}H]spiperone, [^{3}H]noradrenaline hydrochloride, [^{3}H]5-HT binoxalate and [^{3}H]dopamine hydrochloride were obtained from DuPont/NEN Research (Boston, MA, USA). Other chemicals were reagent grade.

2.3. Forced swimming

The forced swimming test was performed according to the method of Porsolt et al. (1977, 1978). Briefly, a Plexiglas cylinder (18 cm, diameter × 40 cm, height) was filled with water warmed at 25°C to the height of 25 cm, and rats were placed in the water for 15 min (first session). On the next day, rats were again forced to swim in the same cylinder for 5 min (second session). The total duration that rats showed a posture of immobility in the second session was measured. Drugs were administered orally immediately after the first session and 1 h before the second session.

2.4. Learned helplessness in the shuttle-box test

Learned helplessness in the shuttle-box test was used as another model of behavioral despair (Murua and Molina, 1991). Briefly, rats were individually exposed to a train of inescapable footshocks (0.5 mA, 10 s duration and 5 s interstimulation interval, 100 times) once a day for 3 days in a Plexiglas chamber (30 × 30 × 30 cm), the floor of which consisted of stainless steel grids spaced 1 cm apart (shuttle-box). On the 4th day, rats were trained, in 40-trials, to perform an active avoidance task in a shuttle-box and the number of escape failures was counted. Drugs were administered orally once daily for 10 days (from 7 days before to the third day of exposure to inescapable shocks).

2.5. Receptor binding assays after repeated oral administration

Rats were repeatedly administered either NS-105 (100 mg/kg per day, p.o.) or desipramine (10 mg/kg per day, p.o.) for 14 days and were killed by cervical dislocation on the day following the last treatment. The brain was quickly removed and the cerebral cortex was dissected on ice. The membrane fractions were obtained as follows: brain tissues were homogenized with 10 vol of 50 mM Tris–HCl buffer (pH 7.4) and centrifuged at 39 000 × g for 20 min. The above procedure was repeated twice and the resultant pellets were re-suspended in the same buffer, then stored at −60°C until assayed. For the $GABA_B$ binding assay, frozen membranes were further treated to avoid the influence of endogenous GABA on binding: stored membranes were re-suspended in 10 volumes of 50 mM Tris–HCl buffer and centrifuged at 39 000 × g for 20 min. This procedure was repeated twice and the final pellets were re-suspended in 50 mM Tris–HCl buffer containing 2.5 mM $CaCl_2$ and 40 μM isoguvacine. β-Adrenoceptors and $5\text{-}HT_2$ receptors were labeled by 0.1–2 nM [^{3}H]dihydroalprenolol (Yamada et al., 1983) and 0.1–2 nM [^{3}H]spiperone (Leysen et al., 1978), respectively. $GABA_B$ receptor binding was assayed according to the method of Bowery et al. (1983) with 10–300 nM [^{3}H]GABA in the presence of 40 μM isoguvacine to prevent binding to $GABA_A$ receptors. The non-specific binding to β-adrenoceptors, $5\text{-}HT_2$ and $GABA_B$ receptors was determined in the presence of 1 μM DL-propranolol, 10 μM methysergide and 100 μM (±)-baclofen, respectively. Protein content was measured by the method of Bradford (1976), using bovine serum albumin as the standard. The K_d and B_{max} values were evaluated by Scatchard analysis. Data were obtained from 8–10 separate experiments determined in duplicate.

2.6. Uptake of monoamines into rat brain membrane preparations

The rat cerebral cortex or striatum was homogenized with 10 vol of 0.32 M sucrose, using a teflon homogenizer. After centrifugation at 1000 × g for 10 min, the supernatant was centrifuged at 17 000 × g for 15 min and the resultant pellet was re-suspended in 10–20 volumes of 0.32 M sucrose and used for the measurement of monoamine uptake. [^{3}H]Noradrenaline uptake was determined according to the method of Gehlert et al. (1995), while [^{3}H]5-HT or [^{3}H]dopamine uptake was assayed by the method of Bennett et al. (1995). Briefly, Krebs bicarbonate solution (NaCl, 110 mM; KCl, 5.9 mM; $MgCl_2$, 1.2 mM; NaH_2PO_4, 1.2 mM; $CaCl_2$, 2.5 mM; D-glucose 11.5 mM; $NaHCO_3$, 25 mM, pH 7.4) containing 10 μM

pargyline, 1 mM ascorbic acid and 0.17 mM ethylenediamine tetraacetic acid disodium salt was added to the membrane preparations and incubated at 37°C for 5 min under continuous gassing with 95% O_2/5% CO_2. After this incubation, radiolabeled ligands were added and the mixture was incubated at 37°C for 10 min. After incubation, the medium was aspirated through a Whatman GF/B filter, and the filter was washed 3 times with ice-cold Krebs bicarbonate solution. The radioactivity associated with the filter was counted. The non-specific uptake of noradrenaline, 5-HT or dopamine was measured in the presence of 10 μM each of maprotiline, fluoxetine or nomifensine, respectively. Specific uptake was calculated as the difference in the radioactivity counts between total and non-specific uptake. The experiments were carried out in duplicate and data were obtained from 4 independent experiments.

2.7. Intracerebral microdialysis

The extracellular concentrations of noradrenaline and 5-HT were measured by intracerebral microdialysis in urethane-anesthetized rats, as described previously (Itoh et al., 1994, 1996). Briefly, a microdialysis probe (I-shaped: BDP-I-4-03, Eicom, Kyoto, Japan) was inserted into the cerebral cortex (3.7 mm anterior to the bregma, 3.0 mm lateral and 3.8 mm below the dura mater) and Ringer's solution (NaCl, 147 mM; KCl, 4 mM; $CaCl_2$, 2.3 mM) was perfused at a flow rate of 2 μl/min. Microdialysates were collected into polyethylene tubes containing 10 μl of 0.1 M formic acid, 2–3 h after the start of perfusion. NS-105 was dissolved in Ringer's solution. Rats were wrapped in a blanket to maintain constant body temperature throughout the experiment. At the end of the experiment, brains were perfused with 50 ml of 10% formalin saline solution via a carotid artery, and serial 50 μm thick coronal brain sections were made to verify the location of the tip of the dialysis probe.

2.8. Determination of monoamines and their metabolites in rat brain regions

The concentrations of noradrenaline and 5-HT in microdialysates were determined by high-performance liquid chromatography (HPLC) with electrochemical detection. The HPLC system consisted of a pump (EP-300, Eicom) equipped with a damper (DG-300, Eicom), a guard column (4.0 × 2.1 mm inside diameter) packed with Nucleosil 5C18 (Chemco), a reversed-phase separation column (CA-50 DS: 150 × 2.1 mm inside diameter, Eicom), an electrochemical detector (ECD-300, Eicom) and a recorder (Chromatopac C-R4A). The potential of the working electrode was set at 0.45 V vs. an Ag/AgCl reference electrode. The mobile phase was a mixture of 0.1 M sodium phosphate buffer (pH 6.0) and methanol (95:5 (v/v) for the measurement of noradrenaline; 84:16 (v/v) for 5-HT assay) containing 10 μM ethylenediamine tetraacetic acid disodium salt and 1.85 mM sodium 1-octanesulfonate. The flow rate was 0.21 ml/min.

For the measurement of monoamines and their metabolites in rat brain tissues, brains were dissected on ice into six regions, namely the cerebral cortex, hippocampus, striatum, hypothalamus, midbrain and pons–medulla oblongata, according to the method of Glowinski and Iversen (1966). The tissues were homogenized with more than 10 vol of 0.4 M perchloric acid containing an appropriate amount of 3,4-dihydroxybenzylamine, as an internal standard. Monoamines and their metabolites in supernatants were determined according to the method of Warnhoff (1984) with modifications (Itoh et al., 1996).

2.9. Statistical analyses

Statistical analyses were performed using SAS program (SAS/STAT, Ver. 6, fourth edition, 1990, SAS Institute

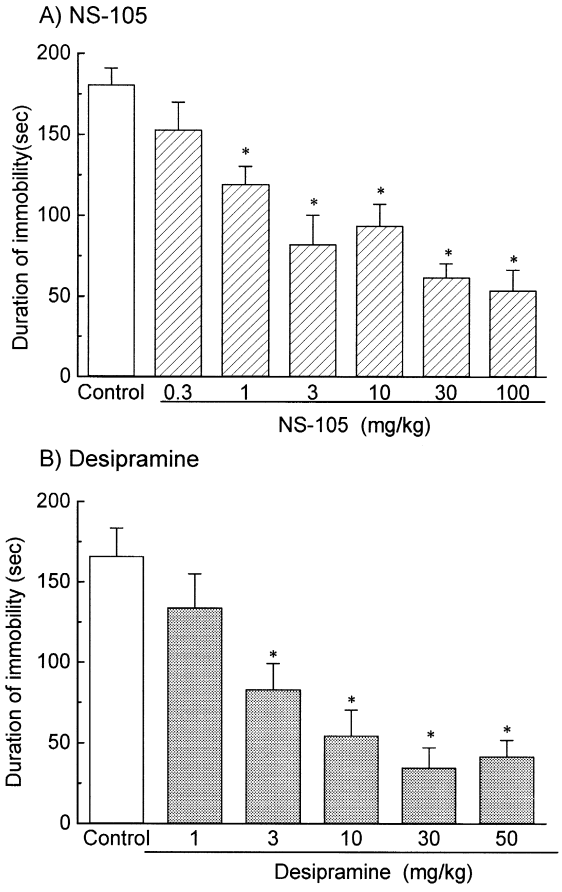

Fig. 1. Effects of NS-105 and desipramine on immobility of rats in the forced swimming test. Rats were forced to swim for 15 min in warmed water (25°C) (first session) and were then exposed to the same situation for 5 min on the second day (second session). The total duration of immobility during the second session was measured. Drugs were administered p.o. immediately after the first session and 1 h before the second session. Each column represents the mean ± S.E.M. for 10 animals. * $P < 0.01$ vs. Control.

Ins., Cary, NC). Data for forced swimming were compared between control and drug-treated groups and analyzed by Dunnett's test. Data obtained for learned helplessness in the shuttle-box test were evaluated by comparing the values of drug-treated groups with those of the saline-treated group. Biochemical data were analyzed by one-way analysis of variance followed by Dunnett's test.

3. Results

3.1. Behavioral actions of NS-105

In the forced swimming test, NS-105 (0.3–100 mg/kg, p.o.) produced a dose-dependent decrease in the duration of immobility, and significant effects were observed at doses higher than 1 mg/kg (Fig. 1). Desipramine (1–50 mg/kg, p.o.) also decreased the duration of immobility in a dose-dependent manner, significant actions being observed at > 3 mg/kg.

When rats were previously exposed to inescapable footshock, they failed to escape in the subsequent active avoidance task in the shuttle-box test (Murua and Molina, 1991). As shown in Fig. 2, repeated administration of either NS-105 (1–30 mg/kg, p.o.) or desipramine (1–10 mg/kg, p.o.) for 10 days reduced the number of escape failures in a dose-dependent manner, and significant effects were observed at doses higher than 10 mg/kg of NS-105 and at 10 mg/kg of desipramine.

NS-105 at the doses tested in the present experiment

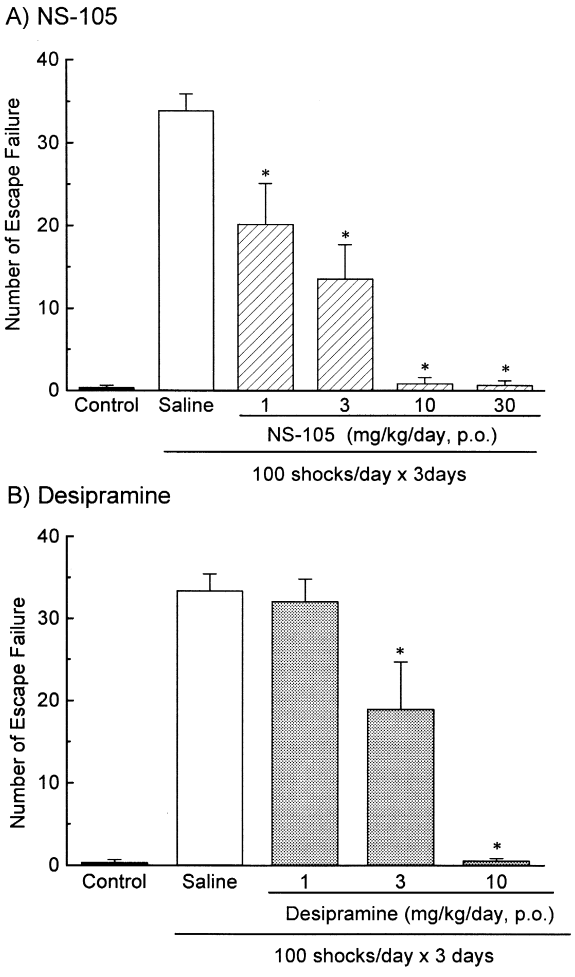

Fig. 2. Effects of NS-105 (A) and desipramine (B) on escape failures in the learned helplessness test in rats. Rats were exposed to inescapable footshock for 3 days and were then subjected to 40-trials of an active avoidance task in a shuttle-box on the following day. The total number of escape failures during the 40 trials was counted. Drugs were administered p.o. once daily for 10 days (from 7 days before to the third day of the exposure to inescapable footshock). Each column represents the mean ± S.E.M. for 10 animals. $^{*}P < 0.01$ vs. Saline treated group.

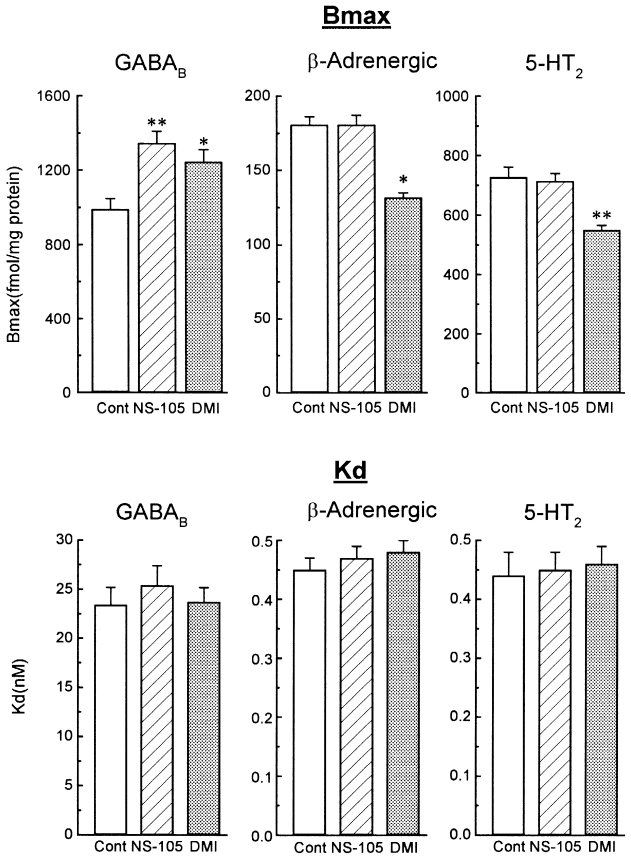

Fig. 3. Effects of repeated administration of NS-105 and desipramine (DMI) on binding properties of $GABA_B$ receptors, β-adrenoceptors and $5\text{-}HT_2$ receptors in rat cerebral cortex. NS-105 (100 mg/kg) or desipramine (10 mg/kg) was administered p.o. once daily for 14 days. Animals were killed 24 h after the last administration, and crude synaptosomal membranes were prepared. $GABA_B$ receptors were labelled with [^{3}H]GABA in the presence of 40 μM isoguvacine to prevent binding to $GABA_A$ receptors. β-adrenoceptors and $5\text{-}HT_2$ receptors were labelled with [^{3}H]dihydroalprenolol and [^{3}H]spiperone, respectively. The K_d and B_{max} values were estimated by Scatchard analysis. Each column represents the mean ± S.E.M. of 8 to 10 experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. Control.

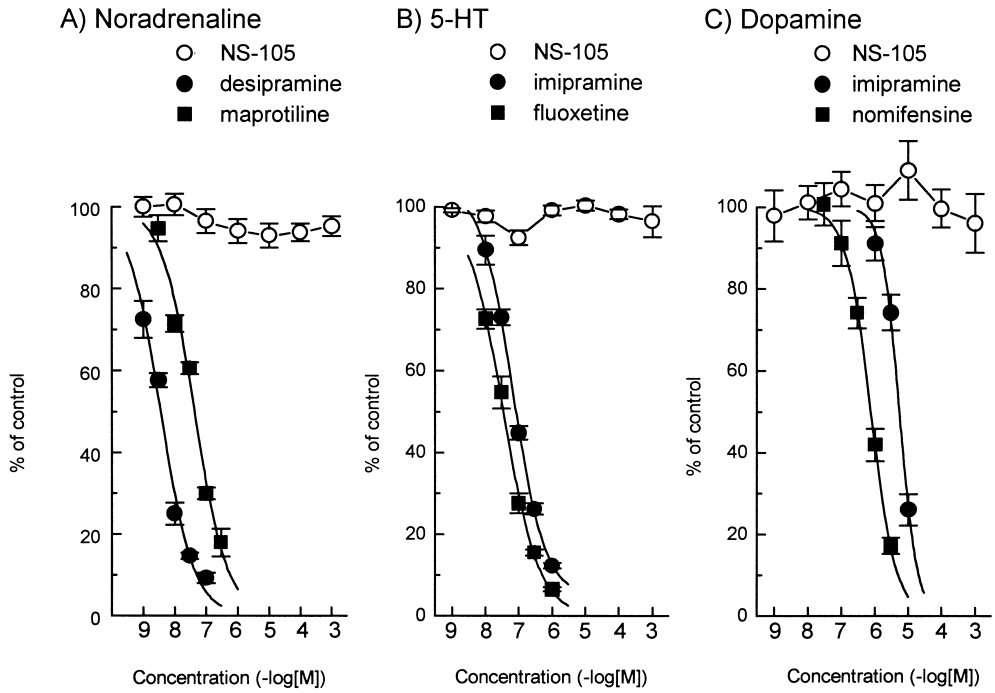

Fig. 4. Effects of NS-105 and various antidepressants on the uptake of [^{3}H]noradrenaline (A), [^{3}H]5-HT (B) and [^{3}H]dopamine (C) into rat brain synaptosomal membranes. The uptake of [^{3}H]noradrenaline and [^{3}H]5-HT was measured in the cerebral cortex, while that of [^{3}H]dopamine was determined in the striatum. Each point represents the mean ± S.E.M. of 4 experiments.

caused no obvious behavioral changes. Locomotor activity was slightly and not significantly lowered after administration of 100 mg/kg of this compound, although a significant ($P < 0.05$) reduction in locomotor activity was observed at 300 mg/kg (the activity counts during 60 min measured by photocell counting were 368 ± 46 in control group, 282 ± 28 in 100 mg/kg NS-105 (100 mg/kg)-treated group, and 225 ± 36 in NS-105 (300 mg/kg)-treated group (mean ± S.E.M. of 6 animals)).

3.2. Effects of repeated administration of NS-105 on binding properties of $GABA_B$ receptors, β-adrenoceptors and 5-HT_2 receptors in rat cerebral cortex

To determine the possible mechanisms underlying the antidepressant action of NS-105, we examined the effect of repeated administration of NS-105 on the binding properties of $GABA_B$ receptors, β-adrenoceptors and 5-HT_2 receptors in rat cortical membranes. As shown in Fig. 3, NS-105 (100 mg/kg, p.o., once daily for 14 days) caused a significant increase in the number (B_{max}) but not the affinity (K_d) of $GABA_B$ receptor without affecting the binding properties of either β-adrenoceptors or 5-HT_2 receptors. Repeated administration of desipramine (10 mg/kg, p.o., once daily for 14 days) also significantly increased the number of $GABA_B$ receptors, although it decreased the number of β-adrenoceptors and 5-HT_2 receptors.

3.3. Effects of NS-105 on monoamine dynamics in rat brain

Unlike other antidepressant drugs, NS-105 (1 nM–1 mM) had no influence on the uptake of [^{3}H]noradrenaline (Fig. 4A), [^{3}H]5-HT (Fig. 4B) and [^{3}H]dopamine (Fig. 4C) into crude synaptosomal membranes. In contrast, desipramine and maprotiline potently inhibited [^{3}H]noradrenaline uptake with IC_{50} values of 3.6 ± 0.6 and 41.0 ± 2.9 nM, respectively (mean ± S.E.M., $N = 4$) (Fig. 4A). [^{3}H]5-HT uptake was strongly inhibited by imipramine and fluoxetine with IC_{50} values of 85.3 ± 7.9 and 35.4 ± 5.5 nM, respectively (Fig. 4B). Both imipramine and nomifensine, a selective dopamine uptake inhibitor, inhibited [^{3}H]dopamine uptake, the IC_{50} values of which were 5.4 ± 0.6 μM and 0.750.13 μM, respectively (Fig. 4C).

Also in the in vivo microdialysis experiment, perfusion of NS-105 (0.1–100 μM) into the cerebral cortex did not affect the extracellular concentrations of noradrenaline (Fig. 5A) and 5-HT (Fig. 5B) in the same brain area, whereas desipramine (0.1–1 μM) and imipramine (1 μM) markedly increased the extracellular concentrations of noradrenaline and 5-HT, respectively. In addition, single oral administration of NS-105 (10–100 mg/kg) did not significantly change tissue concentrations of noradrenaline, dopamine and its metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid, 5-HT and 5-hydroxyindoleacetic acid determined 1 h after injection in the cerebral cortex,

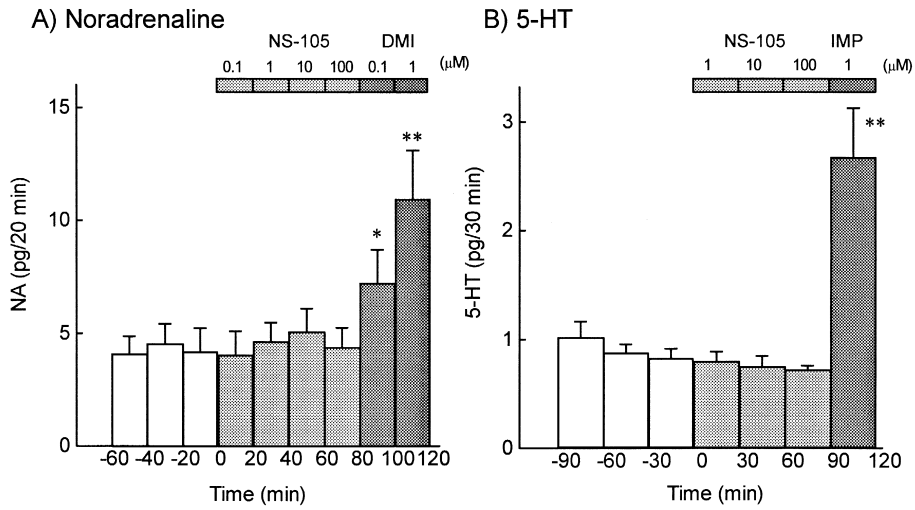

Fig. 5. Effects of NS-105 and tricyclic antidepressants on the extracellular concentrations of noradrenaline (NA) (A) and 5-HT (B) in rat cerebral cortex, as measured by intracerebral microdialysis. Rats were anesthetized with urethane (1.1 g/kg, i.p.) and Ringer's solution was perfused at a flow rate of 2 μl/min. Two to three hours after the start of perfusion, microdialysates were collected every 20 min (in case of noradrenaline) or 30 min (in case of 5-HT). NS-105, desipramine (DMI) or imipramine (IMP) was perfused into the cerebral cortex through the microdialysis probe. Each column represents the mean ± S.E.M. of 5 experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. average of three consecutive pre-drug infusion values.

hippocampus, striatum, hypothalamus, midbrain and pons–medulla oblongata in rats (data not shown).

4. Discussion

The forced swimming test, which was originally developed by Porsolt et al. (1977) as an animal model of behavioral despair, is currently used as a screening model for antidepressants, since several antidepressant drugs reduce the immobility in this behavioral paradigm. However, forced swimming is especially sensitive to antidepressant drugs which activate central catecholaminergic neurons, and drugs which selectively inhibit 5-HT uptake such as fluoxetine and chlorimipramine were less active in this behavioral model (Porsolt et al., 1979). Conversely, several compounds which stimulate locomotor activity, such as amphetamine, apomorphine and caffeine (Porsolt et al., 1978), or those that disrupt learning and memory, such as anticholinergic agents (Herman et al., 1981), have been reported to act as false positives in this behavioral test. Murua and Molina (1991) have reported in another learned helplessness model, in which escape failure in a shuttle-box test is measured in rats with previous exposure to inescapable footshock, that chronic administration of a variety of antidepressants reduces the number of escape failures. In the present experiment, NS-105 was as potent and as effective as desipramine in the two representative screening models for antidepressant drugs. NS-105 at the doses used in the present experiment had no significant influence on locomotor activity. In addition, this compound did not cause amnesia, but it rather reversed the impairment of learning and memory induced by the dysfunction of central cholinergic systems (Nakagawa et al., 1988, 1990; Ukai et al., 1990). Therefore, it is suggested that NS-105 possesses potent antidepressant activity.

In the present experiment, repeated administration of NS-105 significantly increased the number of $GABA_B$ receptors in the rat cerebral cortex. A similar result was obtained after repeated administration of desipramine. Although the precise mechanisms underlying the therapeutic action of antidepressant drugs remain to be clarified, an involvement of $GABA_B$ receptor up-regulation in the antidepressant action has been postulated by several investigators: the B_{max} but not K_i value for $GABA_B$ receptor binding increased in rat cerebral cortex and hippocampus after repeated administration of a variety of antidepressant drugs (Lloyd et al., 1985; Suzdak and Gianutsos, 1986; Motohashi et al., 1989; Pratt and Bowery, 1993) or chronic exposure to electroconvulsive shocks (Lloyd et al., 1985). Therefore, the up-regulation of $GABA_B$ receptors after repeated administration may at least in part contribute to the antidepressant action of NS-105.

However, we do not know the precise reason for the $GABA_B$ receptor up-regulation after chronic treatment with NS-105, since this compound did not affect the binding of various neurotransmitters to $GABA_B$ receptors, α- and β-adrenoceptors, dopamine receptors, 5-HT_2 receptors, muscarinic acetylcholine and $GABA_A$ receptors in rat brain in vitro (Oka et al., 1997). We have recently found that NS-105 reverses baclofen-induced inhibition of adenylate cyclase activity in slices of the rat cerebral cortex via an interaction with GTP-binding proteins (Oka et al., 1997). Pratt and Bowery (1993) have demonstrated in an autoradiographic study that $GABA_B$ receptors are up-regulated after repeated administration of a selective $GABA_B$ receptor antagonist CGP 36742. Conversely, down-regulation of $GABA_B$ receptors is reported to occur after

long-term treatment with baclofen (Suzdak and Gianutsos, 1986). Therefore, it is likely that the functional antagonism by NS-105 against $GABA_B$ receptor-mediated responses, such as inhibition of adenylate cyclase activity, is involved in $GABA_B$ receptor up-regulation after its long-term treatment.

Most antidepressant drugs are known to interact with monoaminergic systems. It has been demonstrated that β-adrenoceptors and $5\text{-}HT_2$ receptors are down-regulated in the cerebral cortex after chronic treatment with a variety of antidepressant drugs (Peroutka and Snyder, 1980; Friedhoff and Miller, 1983; Green et al., 1983; Sulser, 1984; Vetulani et al., 1984; Okada et al., 1986). Also, in the present experiment, repeated administration of desipramine (10 mg/kg, p.o., once a day for 14 days) caused significant decreases in the number of β-adrenoceptors and $5\text{-}HT_2$ receptors. However, repeated administration of NS-105 did not affect the binding properties of either β-adrenoceptors or $5\text{-}HT_2$ receptors. In addition, unlike other antidepressant drugs, NS-105 had no influence on monoamine uptake in vitro and central monoaminergic transmission (release and turnover) in vivo. Therefore, it is unlikely that the antidepressant action of NS-105 is mediated by central monoaminergic neurons.

In conclusion, a pyroglutaminamide derivative NS-105 had potent antidepressant activity in rats in the forced swimming and learned helplessness tests. Repeated treatment with NS-105 caused up-regulation of $GABA_B$ receptors without affecting the dynamics of β-adrenoceptors and $5\text{-}HT_2$ receptors in rat cerebral cortex. Unlike other antidepressants, NS-105 had no influence on either monoamine uptake or central monoaminergic transmission, and thus the compound may have potential in the therapy of depressive illness.

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