Characteristics of the Ambulation-Increasing Effect of GBR-12909, a Selective Dopamine Uptake Inhibitor, in Mice

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ABSTRACT — Behavioral effects of a dopamine uptake inhibitor, GBR-12909 (GBR), were evaluated by ambulatory activity in mice. The single administration of over 10 mg/kg of GBR, i.p. and p.o., significantly increased the ambulatory activity. The repeated administration of GBR, at only 10 mg/kg, produced a reverse tolerance to its ambulation-increasing effect. However, a cross-reverse tolerance was induced between GBR (10 and 20 mg/kg) and methamphetamine (2 mg/kg) in both directions. Furthermore, 5 mg/kg of GBR significantly enhanced the effects of methamphetamine, cocaine, imipramine, morphine, scopoline and caffeine. R-THBP, a coenzyme of tyrosine hydroxylase, also enhanced the effect of GBR. In contrast, the ambulation-increasing effect of 10 mg/kg of GBR was markedly reduced by haloperidol, chlorpromazine, tetrabenazine, oxyppertine, reserpine and a-methyl-p-tyrosine. On the other hand, the effect of GBR was only slightly and/or scarcely modified by apomorphine, caerulein, physostigmine, pilocarpine, N6-(L-2-phenylisopropyl)-adenosine and naloxone. The neurochemical experiment in rats, not in mice, revealed that GBR possessed more dominant action on dopaminergic systems than noradrenergic or serotonergic systems. However, the behavioral characteristics of GBR are similar to those of methamphetamine and cocaine, which possess less selective action than GBR on dopaminergic and noradrenergic systems.

Dopaminergic and noradrenergic neurons in the central nervous system play important roles in the behavioral effects of drugs. Methamphetamine and cocaine are compounds which act as central stimulants through an acceleration of release and/or an inhibition of uptake of dopamine (DA) and norepinephrine (NE) (1). In the animal experiments, methamphetamine and cocaine both increase ambulatory activity at comparatively lower doses, and they produce stereotyped behaviors at higher doses (2). Furthermore, it has been well-known that the repeated administration of methamphetamine or cocaine produces reverse tolerances to its ambulation-increasing and stereotypy-producing effects (3–5). The dopaminergic systems have been considered to play an important role in the development of reverse tolerance to amphetamines and their related drugs (4). There are many DA- and NE-uptake inhibitors, such as nomifensine, methylphenidate and mazindol, and their behavioral effects resemble those of methamphetamine and cocaine (2). However, these compounds lack selectivity for either noradrenergic or dopaminergic neuron.
On the other hand, GBR-12909 (GBR), 1-[2-(bis-4-fluorophenylmethoxy)ethyl]-4-[3-phenylpropyl]piperazine, is a compound with a selective inhibitory action on DA uptake (IC$_{50}$ = 1 nM; the corresponding values for NE and 5-HT uptake are 440 and 170 nM, respectively) (6). An ex vivo study in rats revealed that GBR inhibited DA-uptake with an ED$_{50}$ of 26 mg/kg (i.p. 2 hr before), but it was without effect on NE-uptake. In a behavioral study in mice and rats, GBR was reported to show a stimulant action, eliciting an increase in motor activity, induction of stereotyped behavior (7–10), and an ameliorative effect on learned helplessness (8). The drug discrimination test revealed that although cue-generalization hardly occurred from d-amphetamine to GBR, that in the opposite direction usually took place (8, 11). Furthermore, GBR produced ipsilateral rotation in the rat that had received unilateral lesion of the nigro-striatum (7). In these respects, GBR has been considered to possess behavioral-pharmacological effects that are partially common to those of amphetamines, anti-depressants and/or cocaine. However, the profile of the behavioral effects of this DA uptake inhibitor has been evaluated insufficiently.

The present study was designed to assess the behavioral effects of a DA uptake inhibitor in terms of ambulatory activity by using GBR as a tool in mice. The present study consisted of 3 main types of experiments: 1) observation of the effect of GBR alone after single and repeated administration, 2) observation of the cross-interaction between GBR and methamphetamine, and 3) observation of the combined effects of GBR with various types of drugs. The last experiment was conducted because dopaminergic systems may interact with noradrenergic, cholinergic and adenosine systems in vivo.

MATERIALS AND METHODS

Animals

Male mice of the ddY strain at 6 weeks of age and male rats of the Wistar strain at 8 weeks of age were purchased from Japan Laboratory Animal and Shinnihon Dobutsu, respectively. Ten mice and 5 rats were group housed in standard breeding cages with a wooden-flake floor mat (White-flake: Charles River Japan), and they were freely given solid diet (MF, Oriental Yeast) and tap water except during the times of the experiment. The breeding room was controlled so that the light-dark schedule (lighting time: 06:00–18:00) and room temperature (23 ± 1°C) were almost constant.

Drugs

Drugs used in this study were: hydrochloric form of GBR (Gist-brocades), methamphetamine hydrochloride (Philopon, Dainippon Pharm.), cocaine hydrochloride (Takeda Chem.), imipramine hydrochloride (Tofranil Inj., Ciba-Geigy Japan), morphine hydrochloride (Takeda Chem.), apomorphine hydrochloride (Sigma Chem.), scopolamine hydrobromide (Sigma Chem.), caffeine anhydrous (Kanto Chem.), haloperidol (Serenace Inj., Dainippon Pharm.), chlorpromazine hydrochloride (Cotomin Inj., Yoshitomi Pharm.), tetrabenazine hydrochloride (Pfizer-Taito), oxypertine (Forit, Daiichi Pharm.), reserpine (Apoplon Inj., Daiichi Pharm.), caerulein (Shionogi), 6R-L-erythro-5,6,7,8-tetrahydrobiopterin (R-THBP, Suntoy), a-methyl-tyrosine (Sigma Chem.), physostigmine sulfate (Sigma Chem.), pilocarpine hydrochloride (Sigma Chem.), N$_6$-(L-2-phenylisopropyl)-adenosine (PIA: Sigma Chem.) and naloxone hydrochloride (Sigma Chem.). These drugs were dissolved or suspended in or diluted by physiological saline. The concentration of each drug solution or suspension was adjusted so that each volume administered was constant at 10 ml/kg to mice and 1 ml/kg to rats.

Experimental procedure for ambulatory activity test

The ambulatory activity of the mouse was measured using a tilting-type ambulometer (AMB-10, O’hara). Mice were individually placed in plexiglas activity cages with a diam-
eter of 20 cm, and their ambulatory activities were measured for 30 min and 2–5 hr before and after the drug administration, respectively.

**Single administration of GBR**: Groups of 10–20 mice were given intraperitoneally (i.p.) GBR (2.5, 5, 10 and 20 mg/kg) or physiological saline (10 ml/kg), and their ambulatory activities were measured thereafter for 5 hr.

**Repeated administration and cross administration of GBR and methamphetamine**: Five groups of mice (N = 14–19) were repeatedly treated with GBR (0: physiological saline, 2.5, 5, 10 and 20 mg/kg, i.p.) at intervals of 2–3 days, and each administration was followed by 3-hr measurement of ambulatory activity. All of these mice were cross administered subcutaneously (s.c.) methamphetamine (2 mg/kg) at 2 or 3 days after the 5th treatment with GBR, and their ambulatory activities were measured for 3 hr. The age-matched and drug-naive mice were also given the same dose of methamphetamine.

On the other hand, 5 groups of mice (N = 14–18) were first given the repeated 5 times administration of methamphetamine (2 mg/kg, s.c.) at intervals of 3–4 days to induce reverse tolerance (an increase in susceptibility) to the ambulation-increasing effect. Then these mice were cross-administered GBR (0, 2.5, 5, 10 or 20 mg/kg, i.p.) on 2–4 days after the last dose of methamphetamine.

The doses, administration intervals and number of methamphetamine administrations employed in this test were considered to be appropriate for induction of the reverse tolerance to the ambulation-increasing effect of methamphetamine in mice (3, 5).

**Combined administration of GBR with central-acting drugs**: Ambulatory activity of mice was measured for 2 to 3 hr after the combined administration depending on the duration of each drug effect. The doses of GBR (i.e., 5 and 10 mg/kg) were taken to be optimum doses according to the single administration of GBR in this study, and the doses of central-acting drugs (refer Figs. 5–6) were chosen on the basis of our experience with these drugs.

**Monoamine assay**

The rats were treated with 7 daily administrations of GBR (6.25–50 mg/kg, i.p.) or saline. Two hours after the 7th treatment, the rats were irradiated with microwaves (10 kW, 0.85 sec) and decapitated. The brain was rapidly removed, and the cortex, hippocampus and striatum were separated according to the method of Globinski and Iversen (12).

Regional levels of norepinephrine (NE), dopamine (DA), 3-methoxy-4-hydroxyphenyl glycol (MHPG), 3,4-dihydroxyphenylacetic acid (DOPAC), homovalelic acid (HVA), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were analyzed by HPLC-ECD.

**Statistical analysis**

The mean overall ambulatory activity counts for 2–5 hr after the drug administration and the turnover rates for monoamines were calculated in individual groups of animals. At first, repeated measures analysis of variance of the overall data were examined. If there were significant overall effects, comparisons between individual data were conducted by the two-tailed Student's t-test.

**RESULTS**

**Single administration of GBR**

Figure 1 illustrates the time-course changes in activity counts and overall activity counts for 5 hr after i.p. administration of GBR (0, 2.5, 5, 10 and 20 mg/kg). The ambulatory activity of mice increased in a dose-dependent manner, with a maximal increase at 30 min after the administration. The ambulation-increasing effect lasted for about 3 hr at 10 mg/kg, and for 5 hr at 20 mg/kg. The overall activity counts for 5 hr after the administration of 10 and 20 mg/kg of GBR were significantly higher than the saline-administered control value.

**Repeated administration and cross administration of GBR and methamphetamine**

Figure 2 shows the activity counts for 3 hr after repeated 5 times administration of GBR
Fig. 1. Time-course changes and 5-hr overall ambulatory activity counts after administration of GBR-12909 (GBR: 0 = saline, 2.5, 5, 10 and 20 mg/kg, i.p.) to mice. *: Significantly different from the saline-treated control value (P < 0.05). (•••) saline (N = 20); (●●●) GBR, 2.5 mg/kg (N = 15); (○○○) GBR, 5 mg/kg (N = 15); (▲▲▲) GBR, 10 mg/kg (N = 15); (ΔΔΔ) GBR, 20 mg/kg (N = 15).

Fig. 2. Changes in 3-hr overall activity counts after repeated 5 times administration of saline (SAL) or GBR-12909 (GBR: 2.5, 5, 10 and 20 mg/kg, i.p.) at intervals of 2–3 days. *: Significantly different from the activity counts in the 1st administration within the group (P < 0.05). N = 13–19.
(0, 2.5, 5, 10 and 20 mg/kg). Repeated administration of GBR, at 10 mg/kg, produced a progressive enhancement of the ambulation-increasing effect, and the activity count in the 5th administration was significantly higher (about 1.8 times) than that in the 1st administration. With GBR, at 2.5, 5 and 20 mg/kg, as well as with physiological saline, the activity counts in the 2nd–5th administrations were essentially comparable with those in the corresponding 1st administration.

Figure 3 shows the mean overall activity counts for 3 hr after the cross administration of methamphetamine (2 mg/kg) to the mice that had experienced the repeated 5 times administration of GBR (0, 2.5, 5, 10 and 20 mg/kg). The repeated treatment with saline and with GBR (2.5 and 5 mg/kg) produced no significant change in the sensitivity of mice to the ambulation-increasing effect of methamphetamine, whereas the methamphetamine-sensitivity was significantly augmented by the repeated treatment with 10 and 20 mg/kg of GBR.

Figure 4 shows the activity counts for 3 hr after the cross administration of GBR (0, 2.5, 5, 10 and 20 mg/kg) to the mice that had developed reverse tolerance to the ambulation-increasing effect of methamphetamine. The mean overall activity counts in the 5th administration of methamphetamine ranged between 6,600 and 7,000 counts, which were 3–3.5 times as high as those in the 1st administration (data not shown). Mice that demonstrated reverse tolerance to methamphetamine showed significantly higher activity counts than the drug-naive mice after the cross administration of 10 and 20 mg/kg of GBR.

![Fig. 3](image1.png)

**Fig. 3.** Overall activity counts for 3 hr after the cross-administration of methamphetamine (2 mg/kg, s.c.) to the mice that had treated with repeated 5 times administration of saline or GBR-12909 (GBR: 2.5, 5, 10 or 20 mg/kg, i.p.). The cross-administration was carried out 2–4 days after the 5th dose of GBR. Administration of methamphetamine to the drug-naive mice was also conducted (open column, N = 40). *: Significantly different from the mean activity count of mice that have received saline (dose = 0) (P < 0.05). Numbers of mice used are presented in Fig. 2.

![Fig. 4](image2.png)

**Fig. 4.** Overall activity counts for 3 hr after the cross-administration of GBR-12909 (GBR) to mice that developed reverse tolerance to ambulation-increasing effect of methamphetamine (2 mg/kg, s.c.). Five groups of mice showing mean overall 3-hr activity counts of 2,000–2,500 counts and 6,600 to 7,000 counts in the 1st and 5th dose of methamphetamine, respectively, were cross-administered GBR (0 = saline, 2.5, 5, 10 and 20 mg/kg, i.p.), 3–4 days after the 5th methamphetamine administration. *: Significantly different from the activity count of the drug-naive mice given the same dose of GBR (P < 0.05). N = 14–18. □ drug-naive mice, ■ methamphetamine, 2 mg/kg × 5 experienced mice.
Combined administration of GBR with central-acting drugs

The ambulation-increasing effect of methamphetamine was significantly augmented by 2.5 and 5 mg/kg of GBR (data not shown), although GBR alone caused no significant increase in the activity at these doses as shown in Fig. 1.

Figure 5 illustrates the 2- or 3-hr activity counts of mice following combined administration of GBR (5 mg/kg, i.p.) with methamphetamine (2 mg/kg, s.c.), cocaine (40 mg/kg, s.c.), imipramine (20 mg/kg, i.p.), morphine (10 mg/kg, s.c.), apomorphine (0.5 mg/kg, s.c.), scopolamine (0.5 mg/kg, s.c.) and caffeine (10 mg/kg, s.c.). GBR significantly augmented the ambulation-increasing effects of methamphetamine, cocaine, morphine, scopolamine and caffeine. Although imipramine alone was without effect on the mouse's ambulatory activity, it was markedly increased by the combined administration of GBR with imipramine. GBR scarcely modified the ambulatory-increasing effect of apomorphine.

Figure 6 shows the effects of haloperidol (0.1, 0.2, 0.4 and 0.8 mg/kg, s.c.), chlorpromazine (2 and 4 mg/kg, s.c.), tetrabenazine (4 and 8 mg/kg, s.c.), oxypertine (4 and 8 mg/kg, i.p.), reserpine (0.5 and 2 mg/kg, s.c., pretreatment 4 hr before), caerulein (100 μg/kg, i.p.), R-THBP (100 mg/kg, s.c.), α-methyl-p-tyrosine (100 mg/kg, i.p. × 2, pretreatment 24 and 4 hr before), physostigmine (0.1 mg/kg, s.c.), pilocarpine (4 mg/kg, s.c.), PIA (0.1 mg/kg, s.c.) and naloxone (1 mg/kg, s.c.). The ambulation-increasing effect of GBR was dose-dependently reduced by simultaneous administration of haloperidol, chlorpromazine, tetrabenazine and oxypertine, and by pretreatment with reserpine. Caerulein scarcely modified the ambulation-increasing effect of GBR. The ambulation-increasing effect of GBR was significantly augmented by R-THBP, but significantly reduced by α-methyl-p-tyrosine and PIA. Physostigmine and pilocarpine were effective for reducing the ambulation-increasing effect of GBR, but change in the mean overall activity counts did not attain a significant level. Naloxone scarcely modified the effect of GBR.

Fig. 5. Effects of combined administration of GBR-12909 (GBR: 5 mg/kg, i.p.) with methamphetamine (MAP: 2 mg/kg, s.c.), cocaine (COC: 40 mg/kg, s.c.), imipramine (IMP: 20 mg/kg, i.p.), morphine (MOR: 10 mg/kg, s.c.), apomorphine (AP: 0.5 mg/kg, s.c.), scopolamine (SCP: 0.5 mg/kg, s.c.) and caffeine (CAF: 10 mg/kg, s.c.) on ambulatory activity of mice. Each combined administration of drugs was held simultaneously, and the mouse's ambulatory activities were observed for 2 or 3 hr depending on the duration of action of test drugs. *: Significantly different from the activity count of mice single-administered each test drug (P < 0.05). □ test drug alone. ■ combined with GBR, 5 mg/kg.
Fig. 6. Effects of combined administration of GBR-12909 (GBR: 10 mg/kg, i.p.) with haloperidol (HPD: 0.1, 0.2, 0.4 and 0.8 mg/kg, s.c.), chlorpromazine (CPZ: 2 and 4 mg/kg, s.c.), tetrabenazine (TBZ: 4 and 8 mg/kg, s.c.), oxypertine (OPT: 4 and 8 mg/kg, i.p.), reserpine (RP: 0.5 and 2 mg/kg, s.c. pre-treatment 4 hr before), caerulein (CLN: 100 µg/kg, i.p.), 6R-L-erythro-5,6,7,8-tetrahydrobipterin (R-THBP: 100 mg/kg, s.c.), α-methyl-p-tyrosine (AMPT: 100 mg/kg, i.p. × 2, pre-treatment 24 hr and 4 hr before), physostigmine (PHYSO: 0.1 mg/kg, s.c.), pilocarpine (PILO: 4 mg/kg, s.c.), N⁶-(L-2-phenylisopropyl)-adenosine (PIA: 0.1 mg/kg, i.p.) and naloxone (NX: 1 mg/kg, s.c.) on ambulatory activity of mice. *: Significantly different from the activity count of mice given GBR alone (open column) (P < 0.05). □ GBR, 10 mg/kg, combined with test drug.
Table 1. Effects of GBR-12909 on the ratios between monoamines and their metabolites in 3 regions of rat brain

| Region   | Dose (mg/kg) | MHPG/NE | DOPAC/DA | HVA/DA | 5-HIAA/5-HT |
|----------|--------------|---------|----------|--------|-------------|
| Striatum | 6.25         | 84 ± 26 | 96 ± 4   | 107 ± 7 | 98 ± 6      |
|          | 12.5         | 66 ± 29 | 88 ± 4   | 118 ± 7*| 104 ± 6     |
|          | 25           | 81 ± 21 | 118 ± 11 | 144 ± 5*| 108 ± 8     |
|          | 50           | 74 ± 18 | 146 ± 11*| 205 ± 21*| 116 ± 5*    |
| Cortex   | 6.25         | 117 ± 7 | 102 ± 6  | 92 ± 6  | 98 ± 6      |
|          | 12.5         | 134 ± 30| 105 ± 9  | 92 ± 6  | 104 ± 6     |
|          | 25           | 116 ± 25| 110 ± 9  | 142 ± 13| 103 ± 3     |
|          | 50           | 127 ± 8 | 125 ± 7* | 157 ± 17*| 107 ± 6     |
| Hippocampus | 12.5   | 102 ± 29| 112 ± 17 | 111 ± 7 | 93 ± 6      |
|          | 25           | 115 ± 19| 92 ± 8   | 133 ± 26| 95 ± 6      |
|          | 50           | 153 ± 23| 125 ± 5  | 181 ± 19*| 95 ± 3      |

*P < 0.05. GBR-12909 was administered for 7 days, and the monoamines and their metabolites were analyzed 2 hr after the 7th administration. The data are presented as %s of the saline-treated control values.

Monoamine assay

Table 1 shows the ratios between monoamines and their metabolites in the rat brain after the repeated 7-days administration of GBR. The repeated administration of GBR produced a significant increase in the HVA/DA ratio in the striatum at 12.5 mg/kg and more and significantly increased DOPAC/DA and HVA/DA ratios in the striatum and HVA/DA ratio in the cortex at 50 mg/kg. A significant increase in 5-HIAA/5-HT ratio was produced by 50 mg/kg of GBR in the striatum.

DISCUSSION

GBR has been considered to selectively inhibit DA uptake and to increase the amount of DA at the synapse (6, 9). It has been also reported that GBR facilitates motor activity at low doses and induces stereotyped behavior at high doses in mice and rats (7–10). Consistent with these reports, the present experiment also revealed a significant increase in the ambulatory activity of mice following administration of GBR at 10 mg/kg and more. GBR at 10 mg/kg was considered to be the threshold for increasing the mouse’s ambulatory activity. This consideration may be supported by the results obtained by the repeated administration schedule as well as the cross-interaction with methamphetamine.

Usually, drugs are administered repeatedly in their clinical application. In this respect, it is important to preclinically assess the drug’s effect after repeated administration. An increase in susceptibility (namely reverse tolerance) to the ambulation-increasing effect of GBR was induced when GBR, at only 10 mg/kg, was repeatedly administered, although the activity count in the 5th administration was only about 1.8 times as high as that in the 1st administration. At the other doses, however, neither tolerance nor reverse tolerance was induced. There is a possibility that a development of stereotypy acted to inhibit the enhancement of the ambulation-increasing effect at high doses as suggested by Kelley and Lang (10) in rats. However, a gross observation revealed no marked enhancement of
stereotypy when 20 mg/kg of GBR was repeatedly administered. It is therefore considered that the modification of the effect by the repeated GBR administration attained a ceiling at 20 mg/kg; this characteristic was partially but not absolutely identical with the reverse tolerance to methamphetamine, cocaine and morphine. The reverse tolerance occurs at a relatively wide dose range in the cases of methamphetamine, cocaine and morphine; and the activity counts reach a level 2.5–3.5 times as high as those in the 1st administration (3, 13–15).

Repeated exposure to a drug sometimes leads to alterations in susceptibility to other drugs, i.e., development of cross tolerance and/or cross reverse tolerance. The cross administration tests with GBR and methamphetamine indicated the presence of cross reverse tolerance between two drugs, in both directions. It has been shown in a drug discrimination study that cue-generalization occurs from GBR to d-amphetamine, but not in the reverse direction (8). However, the present results suggest that GBR has CNS actions partially common with those of methamphetamine when the cross-reverse tolerance is taken as the indicator.

The combined administration of GBR with various central-acting drugs yielded results that supported the action of GBR on the dopaminergic and noradrenergic systems.

Some drugs used in this study indirectly activate the dopaminergic and/or noradrenergic systems through facilitation of the release and/or inhibition of the uptake of catecholamines (methamphetamine, cocaine and imipramine) (2), by stimulating the opiate receptors (morphine) (16), by blocking the muscarinic acetylcholine receptors (scopolamine) (17) and by blocking the adenosine receptors (caffeine) (18, 19). GBR was effective for enhancing the ambulation-increasing effects of all these drugs. R-THBP acts as a coenzyme for tyrosine hydroxylase (20), and it markedly enhances the ambulation-increasing effect of methamphetamine (21). This compound also enhanced the effect of GBR. It is therefore curious that the ambulation-increasing effect of apomorphine, a direct DA-receptor agonist (22), was hardly modified by concomitant administration of GBR. There was a possibility that the ambulation-increasing effect of apomorphine (0.5 mg/kg) already attained a ceiling as demonstrated in a previous report (23).

On the other hand, the ambulation-increasing effect of GBR was significantly reduced by blockade of catecholamine receptors (haloperidol and chlorpromazine), catecholamine depletion (tetraubenazine, oxypertine and reserpine) or inhibition of tyrosine hydroxylase (α-methyl-p-tyrosine) (2). These results are consistent with the mechanism of action of GBR that it shows a central-stimulant effect through catecholaminergic systems.

The reductions of GBR’s effect by an indirect and direct agonist on muscarinic cholinergic receptors (physostigmine and pilocarpine) (17) and by an adenosine receptor agonist (PIA) (24) were comparatively smaller than those by antagonists on catecholaminergic receptors. Furthermore, the ambulation-increasing effect of GBR was scarcely modified by an opiate antagonist (naloxone). These findings indicate again that GBR exerts its behavioral effects through catecholaminergic systems.

The neurochemical experiment in rats, but not in mice, suggested that GBR dominantly facilitated the DA turnover, and it inhibited the DA uptake at 12.5 mg/kg, suggesting that the ambulation-increasing effect of GBR is mainly mediated through the action on the dopaminergic systems. Consistent with the previously reported data (6), such neurochemical data for GBR are different from the data on either methamphetamine or cocaine. These two drugs affect DA and NE systems less selectively (25–30). However, the effect of GBR on the ambulatory activity in mice were similar in many respects to those of methamphetamine and cocaine which were previously observed in our study (c.f. 5). Thus, the present results indicate that the neurochemical differences among drugs are not always correlated with their behavioral dif-
ferences. However, our experiment also demonstrated partial differences in the behavioral profiles among GBR, methamphetamine and cocaine. The ambulation-increasing effect of GBR was scarcely modified by caerulein which possesses a marked anti-amphetamine activity (31), indicating that a change in the DA release induced by GBR is almost negligible. Reserpine markedly reduced the effect of GBR, although reserpine scarcely antagonized but sometimes enhanced the methamphetamine-induced behavioral stimulation (25). On the other hand, R-THBP enhanced the ambulation-increasing effect of GBR, similar to methamphetamine. We have demonstrated that R-THBP produces no significant change in the effect of cocaine (21). Furthermore, the modifications by various drugs of the ambulation-increasing effect of GBR suggest that GBR may interact, probably indirectly, with noradrenergic, cholinergic and adenosine systems, etc.

The DA uptake inhibitors are expected to show anti-parkinsonism and anti-depressant activity. When these compounds are clinically applied, side effects which are similar to those produced by methamphetamine and cocaine should be carefully evaluated.

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