Persistent Antagonism of Methamphetamine-Induced Dopamine Release in Rats Pretreated with GBR12909 Decanoate

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ABSTRACT
Methamphetamine abuse is a serious global health problem, and no effective treatments for methamphetamine dependence have been developed. In animals, the addictive properties of methamphetamine are mediated via release of dopamine (DA) from nerve terminals in mesolimbic reward circuits. At the molecular level, methamphetamine promotes DA release by a nonexocytotic diffusion-exchange process involving DA transporter (DAT) proteins. We have shown that blocking DAT activity with high-affinity DA uptake inhibitors, such as 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) piperazine (GBR12909), can substantially reduce amphetamine-induced DA release in vivo. In the present study, we examined the ability of a long-acting depot formulation of GBR-decanoate (GBR-decanoate) to influence neurochemical actions of methamphetamine in the nucleus accumbens of rats. Rats received single injections of GBR-decanoate (480 mg/kg i.m.) and were subjected to in vivo microdialysis testing 1 and 2 weeks later. Pretreatment with GBR-decanoate produced modest elevations in basal extracellular levels of DA, but not 5-hydroxytryptamine (5-HT), at both time points. GBR-decanoate nearly eliminated the DA-releasing ability of methamphetamine (0.3 and 1.0 mg/kg i.v.) for 2 weeks, whereas methamphetamine-induced 5-HT release was unaffected. Autoradiographic analysis revealed that GBR-decanoate caused long-term decreases in DAT binding in the brain. Our data suggest that GBR-decanoate, or similar agents, may be useful adjuncts in treating methamphetamine dependence. This therapeutic strategy would be especially useful for noncompliant patient populations.

Methamphetamine abuse is a growing problem in the United States (Anglin et al., 2000), and no effective treatments for methamphetamine dependence have been found. The powerful addictive properties of methamphetamine are believed to involve release of dopamine (DA) from nerve cells in the brain by a mechanism dependent upon DA transporter (DAT) proteins (Wise, 1996). Based on this information we have examined high-affinity DAT ligands, such as the DA uptake blocker GBR12909, as potential therapeutic agents and as tools to test the DA hypothesis of addiction (Rothman, 1990; Rothman and Glowa, 1995). Previous findings show that pretreatment with GBR12909 suppresses cocaine self-administration behavior in rats and monkeys (Glowa et al., 1995; Tella, 1995). Moreover, GBR12909 antagonizes the DA-releasing ability of amphetamine in rats and baboons (Baumann et al., 1994; Vilelmagne et al., 1999). The preclinical data suggest that GBR12909 might be a useful pharmacological adjunct in the management of stimulant addictions, and clinical safety trials with this medication are underway (Preti, 2000).

There are numerous obstacles to successful treatment of stimulant-dependent patients, including high drop-out rates and lack of compliance with medication regimens (Battjes et al., 1999). To circumvent issues of patient noncompliance, we developed a long-acting depot formulation of GBR12909, 1-[2-[bis(4-fluorophenyl) methoxy]ethyl]-4-(3-hydroxy-3-phenylpropyl) piperazinyl decanoate, or GBR-decanoate, that can be administered as a single i.m. injection (Glowa et al., 1996; Lewis et al., 1999). Figure 1 shows the chemical structure of GBR12909, along with its hydroxy and decanoate

ABBREVIATIONS: DA, dopamine; DAT, dopamine transporter; GBR12909, 1-[2-[bis(4-fluorophenyl) methoxy]ethyl]-4-(3-phenylpropyl) piperazine; GBR-decanoate, GBR12909 decanoate; GBR-hydroxy, 1-[2-[bis(4-fluorophenyl) methoxy]ethyl]-4-(3-hydroxy-3-phenylpropyl) piperazine; RTI-55, 3β-(4-iodophenyl) tropan-2β-carboxylic acid methyl ester; 5-HT, serotonin; SERT, 5-hydroxytryptamine transporter; HPLC, high-pressure liquid chromatography; METH, methamphetamine.
analogs. In a preliminary report, Glowa et al. (1996) showed that one dose of GBR-decanoate suppresses cocaine self-administration behavior in rhesus monkeys for up to 1 month. Like other depot medications (Dreyfuss et al., 1976; Florence and Vezin, 1981), GBR-decanoate is an oil-soluble prodrug that slowly releases an active hydroxylated derivative, 1-[2-(bis(4-fluorophenyl)methoxy)ethyl]-4-(3-hydroxy-3-phenylpropyl) piperazine, or GBR-hydroxy, into the circulation. GBR-hydroxy is a potent and selective inhibitor of DAT binding, similar to GBR12909 itself; in vitro experiments using [\(^{125}\)I]RTI-55 to label DATs and 5-HT transporters (SERTs) demonstrate that GBR-hydroxy displays high affinity for DAT (\(K_i = 2.2 \pm 0.1\) nM) and much lower affinity for SERT (\(K_i = 117 \pm 7\) nM). Accordingly, GBR-hydroxy blocks \(^{3}\)H]DA uptake (\(IC_{50} = 5.6 \pm 0.1\) nM) with greater potency than \(^{3}\)H]5-HT uptake (\(IC_{50} = 69 \pm 7\) nM) (Lewis et al., 1999).

With regard to the above-mentioned findings, we were interested in the potential utility of GBR-decanoate as a medication for methamphetamine dependence. In the present study, in vivo microdialysis methods were used to examine the neurochemical effects of GBR-hydroxy and GBR-decanoate in the nucleus accumbens of conscious rats. In particular, we assessed the ability of the GBR compounds to influence the transmitter-releasing action of methamphetamine. Dialysate samples were assayed for DA and 5-HT using high-pressure liquid chromatography (HPLC) with electrochemical detection. Our data show that acute pretreatment with GBR-hydroxy antagonizes methamphetamine-evoked DA release without affecting 5-HT release. More importantly, a single injection of GBR-decanoate nearly eliminates the DA-releasing capability of methamphetamine for at least 2 weeks. The neurochemical effects of GBR-decanoate seem to involve long-term blockade of DAT sites in the brain. Taken together, the findings suggest that GBR-decanoate could be a useful pharmacological adjunct for treating methamphetamine dependence.

Materials and Methods

Animals. Male Sprague-Dawley rats (Charles River Laboratories, Inc., Wilmington, MA) weighing 300 to 350 g were housed in standard vivarium conditions (lights on from 7:00 AM to 7:00 PM) with food and water freely available. Animals were maintained in facilities fully accredited by the American Association of the Accreditation of Laboratory Animal Care, and experiments were performed in accordance with the Institutional Animal Care and Use Committee of the National Institute on Drug Abuse, Division of Intramural Research.

Chemicals. Sodium pentobarbital was purchased from Sigma-Aldrich (St. Louis, MO), whereas methoxyflurane (Metofane) was purchased from Pittman-Moore (Phillipsburg, NJ). GBR-decanoate was prepared by esterification of the hydroxyl analog of GBR12909, which we abbreviate GBR-hydroxy (Lewis et al., 1999). (+)-Methamphetamine HCl was generously provided by National Institute on Drug Abuse Drug Supply (Rockville, MD). [\(^{125}\)I]RTI-55 (specific activity = 2200 Ci/mmol), also known as [\(^{125}\)I]CFT, was prepared as described previously (Rothman et al., 1998). All other reagents were purchased from Sigma-Aldrich.

In Vivo Microdialysis Methods. Rats received sodium pentobarbital (60 mg/kg i.p.) for surgical anesthesia. An indwelling jugular catheter made of Silastic Medical Grade tubing (Dow Corning, Midland, MI) was implanted. Each rat was then placed in a stereotaxic apparatus, and a plastic intracerebral guide cannula (CMA/Microdialysis, Acton, MA) was implanted 2 mm above the nucleus accumbens (ML, ±1.4 mm and AP, +1.6 mm from bregma; DV, +6.2 mm from dura) according to published methods (Baumann et al., 2000). The guide cannulae were positioned so that microdialysis probes would reside within the core region of the nucleus accumbens, close to the lateral border of the medial shell region. The guide cannula was fixed to the skull using stainless steel screws and dental acrylic. Animals were singly housed postoperatively and allowed 7 to 10 days to recover before any experimental manipulation. On the evening before an experiment, rats were moved to the testing room and lightly anesthetized with Metofane. A microdialysis probe with a 2 × 0.5 mm exchange surface (CMA/12; CMA/Microdialysis) was lowered into the guide cannula, and an extension tube (polyethylene-50) was attached to the jugular catheter. Each rat was placed into its own plastic container and connected to a tethering system that allowed motor activity within the container. The microdialysis inflow and outflow tubing, as well as the catheter extension tubing, was connected to a fluid swivel (Instech Laboratories, Inc., Plymouth Meeting, PA). Artificial Ringer’s solution containing 147.0 mM NaCl, 4.0 mM KCl, and 1.8 mM CaCl₂ was pumped through the probe overnight at 0.5 µl/min. On the next morning, 10-µl dialysate samples were collected at 20-min intervals. Samples were immediately assayed for DA and 5-HT by HPLC with electrochemical detection. When three stable baseline samples were obtained, drug treatments were administered.

Analysis of DA and 5-HT in Microdialysates. Aliquots of the dialysate (5 µl) were injected onto a microbore HPLC C₁₈ column (3 µm, 100 × 1 mm, SepStik; Bioanalytical Systems, Inc., West Lafayette, IN) that was coupled to an amperometric detector (model LC-4C; Bioanalytical Systems). A glassy carbon electrode was set at a potential of +650 mV relative to Ag/AgCl reference. Mobile phase consisting of 150 mM monochloroacetic acid, 150 mM NaOH, 2.5 mM sodium octanesulfonic acid, and 250 µM disodium EDTA, with 1 ml of triethylamine, 6% MeOH, and 6% CH₃CN per liter of water (final pH 5) was pumped (model 260D; ISCO, Lincoln, NE) at a rate of 60 µl/min. Chromatographic data were acquired on-line and exported to a MAXIMA 820 software system (Waters, Milford, MA) for peak amplification, integration, and analysis. Standards of DA and 5-HT were run daily before dialysate samples, and standard curves were linear over a wide range of concentrations (1–1000 pg). A monomeric standard mix containing DA, 5-HT, and their respective acid metabolites was injected before and after the experiment to ensure validity.
of the constituent retention times. Peak heights of unknowns were compared with peak heights of standards and the lower limit of detection (3 × baseline noise) was 100 fg/5-μl sample.

**Experimental Protocol.** For acute experiments with GBR-hydroxy, rats undergoing microdialysis testing received single i.v. injections of drug or saline vehicle. The effects of GBR-hydroxy alone on extracellular DA and 5-HT were examined first. Then, we assessed the ability of pretreatment with GBR-hydroxy (0.5 mg/kg i.v.) to affect methamphetamine-induced stimulation of DA and 5-HT release. For long-term experiments with GBR-decanoate, rats received single i.m. injections of GBR-decanoate (1 ml/kg of 48% solution, or 480 mg/kg) or sesame oil vehicle. Groups of rats were subjected to microdialysis testing 6 to 7 days later (1 week) and 13 to 14 days later (2 weeks). Separate groups of rats were used for the 1- and 2-week experiments. On the day of test, rats received a challenge injection of methamphetamine (0.3 mg/kg i.v.) or saline, followed by a second injection of methamphetamine (1.0 mg/kg i.v.) or saline 1 h later. Microdialysate samples were collected throughout the experiment until 1 h after the second methamphetamine injection.

**Autoradiography.** Autoradiographic methods were used to assess the effects of GBR-decanoate on transporter binding in brain tissue. Rats received single i.m. injections of GBR-decanoate (1 ml/kg of a 48% solution, or 480 mg/kg) or sesame oil vehicle. Separate groups of rats were sacrificed 6 to 7 days later (1 week) and 13 to 14 days later (2 weeks). Brains were removed, frozen in isopentane, and stored at −80°C. DAT and SERT sites were visualized in 30-μm sections of brain tissue using minor modifications of published methods (Staley et al., 1994; Telia et al., 1996). Briefly, slide-mounted sections were placed in Lipshaw racks and preincubated in binding buffer. Slides were dried, desiccated overnight, and apposed to radiosensitive film (Hyperfilm; Amersham Biosciences, Piscataway, NJ) for 4 to 8 days. A Macintosh Apple Power G3 computer and a scanner were used to digitize the sections from film. The NIH Image 1.62 program (available on the Internet at http://rsb.info.nih.gov/nih-image) was used to construct standard curves and quantify relative optical densities in discrete brain regions.

**Data Analysis.** For the microdialysis experiments, the first three samples collected before any treatment were considered baseline, and all subsequent monoamine measures were expressed as a percentage of this baseline. Data were evaluated by one-way (acute drug treatment) or two-way (pretreatment × acute treatment) analysis of variance. For the autoradiography experiments, data were analyzed by one-way (pretreatment) analysis of variance. When significant F values were obtained, Newman-Keul’s post hoc tests were performed to compare group means. P < 0.05 was chosen as the minimum criterion for statistical significance.

**Results**

Figure 2 shows that i.v. administration of GBR-hydroxy (0.3 and 1.0 mg/kg) increased extracellular DA in a dose-dependent manner \(F(2,12) = 5.07, P < 0.02\). As observed previously with GBR12909, GBR-hydroxy produced a modest and long-lasting rise in dialysate DA (Baumann et al., 1994) without changing levels of 5-HT \(F(2,12) = 0.33, P < 0.72\). The sustained elevation in extracellular DA produced by GBR-hydroxy and GBR12909 is consistent with in vitro data, demonstrating that these agents display persistent (i.e., pseudo-irreversible) binding to DAT sites in rat brain (Rothman et al., 1991; Rothman and Glowa, 1995).

The data in Fig. 3 illustrate that i.v. administration of methamphetamine (0.1, 0.3, and 1.0 mg/kg) caused dose-related increases in extracellular DA \(F(3,16) = 31.45, P < 0.00001\) and 5-HT \(F(3,16) = 17.50, P < 0.00001\) in rat nucleus accumbens. It is important to note the difference between the “spike-like” profile of methamphetamine-induced transmitter release (Fig. 3) versus the sustained “plateau-like” elevations in dialysate DA produced by GBR analogs (Fig. 2). The next experiment tested the ability of GBR-hydroxy to alter methamphetamine-evoked DA and 5-HT release. Groups of rats received either i.v. saline or GBR-hydroxy (0.5 mg/kg) at time 0, followed by i.v. saline or methamphetamine (0.5 mg/kg) 60 min later. As depicted in Fig. 4, GBR-hydroxy alone increased extracellular DA about 2-fold compared with saline [main effect of GBR, \(F(1,16) = 11.66, P < 0.0035\)]. Methamphetamine produced marked elevations in dialysate DA levels in saline-pretreated rats [main effect of methamphetamine, \(F(1,16) = 88.8, P < 0.0001\)], and GBR-hydroxy significantly reduced this action of methamphetamine [GBR × methamphetamine interaction, \(F(1,16) = 42.83, P < 0.0001\); Fig. 4, bottom left]. Post hoc analysis revealed that in rats receiving GBR-hydroxy, methamphetamine did not significantly elevate dialysate DA levels above the effects of GBR-hydroxy itself. GBR-hydroxy alone had no effect on extracellular 5-HT \(F(1,16) = 0.52, P < 0.48\). Methamphetamine caused a dramatic rise in dialysate 5-HT \(F(1,16) = 40.3, P < 0.0001\), and this effect was not altered by GBR-hydroxy pretreatment [GBR × methamphetamine interaction, \(F(1,16) = 1.71, P < 0.21\); Fig. 4, bottom right].

Having established that acute administration of GBR-hy-
droxy antagonizes DA release produced by methamphetamine, we conducted microdialysis experiments to test the long-term effects of GBR-decanoate on methamphetamine-induced elevations of extracellular DA and 5-HT. Rats received GBR-decanoate or its oil vehicle, and groups of rats were subjected to microdialysis testing 1 and 2 weeks later. At the 1-week time point, rats pretreated with GBR-decanoate displayed significant 2.5-fold elevations in basal extracellular DA (4.80 ± 1.01 nM; n = 13 rats) compared with oil-treated controls (1.93 ± 0.33 nM; n = 14 rats). As depicted in Fig. 5, i.v. methamphetamine increased dialysate DA levels at 0.3 mg/kg [F(1,23) = 14.92, P < 0.001] and 1.0 mg/kg [F(1,23) = 17.84, P < 0.001] in oil-pretreated controls. GBR-decanoate pretreatment significantly reduced methamphetamine-evoked DA release at 0.3 mg/kg [GBR × methamphetamine interaction, F(1,23) = 6.14, P < 0.02] and 1.0 mg/kg [F(1,23) = 7.70, P < 0.01] doses (Fig. 5, bottom left). In contrast, GBR-decanoate failed to alter methamphetamine-induced increases in 5-HT in the same animals. It should be noted that GBR-decanoate had no effect on baseline levels of extracellular 5-HT.

At the 2-week time point, rats pretreated with GBR-decanoate still displayed significant 2.4-fold elevations in basal extracellular DA (3.95 ± 0.64 nM; n = 14) compared with oil-treated controls (1.69 ± 0.27 nM; n = 14). As depicted in Fig. 6, GBR-decanoate significantly reduced DA release produced by methamphetamine at 0.3 mg/kg [GBR × methamphetamine interaction, F(1,23) = 11.43, P < 0.0025] and 1.0 mg/kg [F(1,23) = 10.29, P < 0.004] challenge doses of methamphetamine (Fig. 6, bottom left). As observed at 1 week, GBR-decanoate did not alter baseline levels of extracellular 5-HT and failed to alter methamphetamine-induced increases in 5-HT (Fig. 6, bottom right).

To evaluate the possible role of DAT sites in mediating the neurochemical consequences of GBR-decanoate, we determined the effects of the drug on DAT binding sites in the brain using autoradiographic methods. Figure 7 demonstrates that a single injection of GBR-decanoate significantly reduced [125I]RTI-55-labeled DAT binding by 30 to 40% in DA-rich regions such as the caudate nucleus, nucleus accumbens, and olfactory tubercle; the reduction in DAT binding was present for at least 2 weeks. No changes in SERT binding were noted between GBR-decanoate- and oil-treated rats at either time point (data not shown). Previous experience with GBR12909 suggests that such decreases in DAT binding are probably due to persistent occupation of DATs by GBR-hydroxy rather than drug-induced down-regulation DAT.

Fig. 3. Effects of i.v. methamphetamine (METH) on extracellular DA (left) and 5-HT (right) in rat nucleus accumbens. Doses of 0.1, 0.3, and 1.0 mg/kg METH were injected at 0, 60, and 120 min, respectively. Data are mean ± S.E.M. for n = 6 rats, expressed as percentage of baseline. Baseline levels of dialysate DA and 5-HT were 1.86 ± 0.37 and 0.23 ± 0.02 nM, respectively. *, P < 0.05 with respect to preinjection control levels.

Fig. 4. Effects of GBR-hydroxy (GBR-OH) on METH-induced DA release (left) and 5-HT release (right) in rat nucleus accumbens. Rats were pretreated with i.v. GBR-OH (0.5 mg/kg) or vehicle at time 0, followed by acute i.v. injection of 0.5 mg/kg METH or saline at 60 min. Data are mean ± S.E.M. for n = 5 rats/group, expressed as percentage of baseline. Baseline levels of dialysate DA and 5-HT were 2.22 ± 0.18 and 0.37 ± 0.11 nM, respectively. *, P < 0.05 with respect to saline-treated group that received METH.

Fig. 5. Effects of GBR-decanoate on METH-induced DA release (left) and 5-HT release (right) in rat nucleus accumbens. Pretreatment with GBR-decanoate (0.5 mg/kg) or vehicle at time 0, followed by acute i.v. injection of 0.5 mg/kg METH at 60 min. Data are mean ± S.E.M. for n = 5 rats/group, expressed as percentage of baseline. Baseline levels of dialysate DA and 5-HT were 4.80 ± 1.01 and 0.37 ± 0.02 nM, respectively.
A major aim of the present study was to characterize neurochemical effects of a depot formulation of GBR12909, GBR-decanoate. We were particularly interested in the ability of this drug to influence methamphetamine-induced neurotransmitter release. GBR12909 is a high-affinity DA uptake inhibitor that is being tested in humans as a medication for stimulant dependence (Preti, 2000), and the decanoate derivative was synthesized in an attempt to generate sustained pharmacological activity in vivo (Lewis et al., 1999). Our findings demonstrate several important consequences of GBR-decanoate treatment in rats, namely, 1) elevation of baseline levels of extracellular DA, 2) suppression of methamphetamine-evoked DA release, and 3) reduction in $[125I]$RTI-55-labeled DAT binding. The dopaminergic effects produced by GBR-decanoate are persistent, lasting for at least 2 weeks after a single injection. Moreover, the effects seem to be DA-specific because no changes in 5-HT neurotransmission were noted. The long-lasting actions of GBR-decanoate are presumably mediated by slow and continuous release of bioactive GBR-hydroxy into the circulation, with subsequent binding of GBR-hydroxy to DAT sites in the brain. In agreement with this notion, the acute neurochemical effects of GBR-hydroxy are similar to the persistent effects of GBR-decanoate.

Fig. 5. Effects of GBR-decanoate (GBR-dec) on METH-induced DA release (left) and 5-HT release (right) in the nucleus accumbens of conscious rats: 1-week test. Rats were treated with single i.m. injections of GBR-dec (1 ml/kg of a 48% solution, or 480 mg/kg) or sesame oil vehicle. One week later, acute i.v. doses of 0.3 and 1.0 mg/kg METH (bottom) were injected at 0 and 60 min, respectively. Acute i.v. saline was administered on the same schedule (top). Data are mean ± S.E.M. for $n = 6$ to 7 rats/group expressed as percentage of baseline. Baseline dialysate DA levels for GBR-dec and vehicle groups were $4.80 ± 1.01$ and $1.93 ± 0.33$ nM. Baseline dialysate 5-HT levels for GBR-dec and vehicle groups were $0.44 ± 0.05$ and $0.41 ± 0.09$ nM. *, $P < 0.05$ with respect to vehicle-pretreated group that received METH.

Fig. 6. Effects of GBR-decanoate (GBR-dec) on METH-induced DA release (left) and 5-HT release (right) in the nucleus accumbens of conscious rats: 2-week test. Rats were treated with single i.m. injections of GBR-dec (1 ml/kg of a 48% solution, or 480 mg/kg) or sesame oil vehicle. Two weeks later, acute i.v. doses of 0.3 and 1.0 mg/kg METH (bottom) were injected at 0 and 60 min, respectively. Acute i.v. saline was administered on the same schedule (top). Data are mean ± S.E.M. for $n = 6$ to 7 rats/group, expressed as percentage of baseline. Baseline dialysate DA levels for GBR-dec and vehicle groups were $3.95 ± 0.64$ and $1.69 ± 0.17$ nM. Baseline dialysate 5-HT levels for GBR-dec and vehicle groups were $0.41 ± 0.09$ and $0.33 ± 0.05$ nM. *, $P < 0.05$ with respect to vehicle-pretreated group that received METH.

(Rothman et al., 1991; Rothman and Glowa, 1995; Tella et al., 1996; Kunko et al., 1997).
The modest and sustained elevation of baseline extracellular DA produced by GBR-decanoate is consistent with the ability of GBR-hydroxy to bind pseudo-reversibly to DAT sites, thereby causing prolonged inhibition of DA reuptake (Rothman et al., 1991; Lewis et al., 1999). As depicted in Fig. 2, acute i.v. injection of GBR-hydroxy causes a slow and steady rise in extracellular DA that reaches a plateau; this effect is reminiscent of the increase in dialysate DA evoked by i.v. GBR12909 (Baumann et al., 1994). A persistent increase in baseline extracellular DA could be therapeutically relevant with regard to treating stimulant dependence. Preclinical evidence indicates that chronic administration of stimulants such as cocaine and methamphetamine causes central DA dysfunction (Seiden et al., 1993; Kuhar and Pilotte, 1996). Indeed, high doses of methamphetamine induce neurotoxic depletions of DA in the brains of nonhuman primates and people (Wilson et al., 1996; Villemagne et al., 1998). Symptoms of stimulant withdrawal in humans include anhedonia, depression, and suicidal ideation, which might reflect deficits in brain DA (Gawin, 1991; Kaleystein et al., 2000). GBR-decanoate, by increasing basal synaptic DA in the brain, might ease the dysphoria of stimulant withdrawal and correct a hypodopaminergic state.

Perhaps the most striking feature of GBR-decanoate treatment is the ability of the drug to virtually eliminate methamphetamine-induced DA release in a persistent manner (e.g., Figs. 5 and 6). It is noteworthy that this effect is DA-specific because methamphetamine-induced 5-HT release is unaffected. From a molecular perspective, methamphetamine stimulates the release of monoamine transmitters (i.e., DA, 5-HT, and norepinephrine) by a nonexocytotic diffusion-exchange mechanism involving transporter proteins in nerve cell membranes (Rudnick and Clark, 1993; Wall et al., 1995). The in vivo microdialysis data presented in Fig. 3 show that acute methamphetamine administration evokes marked dose-related elevations in both DA and 5-HT in rat nucleus accumbens, and this observation agrees with the work of others (Kuczenski et al., 1995; Melega et al., 1995). Transporter proteins, such as DAT and SERT, play a pivotal role in mediating the neurochemical effects of methamphetamine because these proteins serve as gateways for the passage of methamphetamine into cells in exchange for transmitter molecules that flow out. We have shown previously that blocking DAT sites with GBR12909 can prevent the DA-releasing action of methamphetamine in vitro (Rothman et al., 2000). The present data demonstrate that GBR-hydroxy and GBR-decanoate can selectively antagonize methamphetamine-induced DA release in vivo.

Because DA release in the nucleus accumbens is implicated in the addictive properties of methamphetamine (Wise, 1996), it seems possible that GBR-decanoate might reduce the positive reinforcing properties of illicit stimulants. Consistent with this notion, GBR12909 and GBR-decanoate are known to reduce cocaine self-administration in rats and monkeys (Glowa et al., 1995, 1996; Tella, 1995). More recent findings from Glowa et al. (2001) show that GBR12909 also reduces methamphetamine self-administration in monkeys. Stimulant dependence, like other substance use disorders, is a chronic relapsing disease. Methamphetamine addicts in treatment are often poorly compliant and have high drop-out rates (Battjes et al., 1999; Maglione et al., 2000). Similar problems with schizophrenic patients have been addressed in part by the development of long-acting depot preparations of antipsychotic medications, such as haloperidol decanoate (Davis et al., 1993). Based on the data from the present study, it seems plausible that methamphetamine addicts who take GBR-decanoate as a medication may not experience the reinforcing effects of methamphetamine in the event of relapse. Under such circumstances, methamphetamine abuse would be predicted to extinguish.

Our neurochemical data may shed light on the specific mechanisms whereby GBR12909 and GBR-decanoate suppress ongoing stimulant self-administration. We hypothesize that GBR-decanoate reduces stimulant self-administration by a mechanism involving both DA and 5-HT systems. First, by blocking methamphetamine-evoked DA release, GBR-decanoate may reduce positive reinforcing effects of this stimulant as described above. Second, because GBR-decanoate does not alter 5-HT transmission, the serotonergic effects produced by stimulant drugs remain unimpeded (Figs. 4–6). It is well accepted that pharmacological treatments leading to increased synaptic 5-HT are not reinforcing. For example, increased serotonergic transmission decreases brain stimulation reward (Harrison and Markou, 2001), produces conditioned place aversions (Marona-Lewicka et al., 1996), and reduces the positive reward value of stimulants both in animals and humans (Brauer et al., 1996; Rea et al., 1998). Thus, in the presence of GBR-type compounds, illicit stimulants such as methamphetamine become very effective 5-HT-releasing agents that lack positive reinforcing qualities. On the other hand, several studies in rats have shown that pretreatment with monoamine reuptake inhibitors can en-
hance the discriminative stimulus properties of cocaine (Cunningham and Callahan, 1991; Kleven and Koek, 1998), suggesting the possibility that GBR-decanoate might actually amplify the effects of illicit stimulants. Only carefully controlled studies examining the effects of GBR compounds in people will resolve this issue.

A fundamental premise of the present study is that the effects of GBR-decanoate are mediated by a slow release of GBR-hydroxy into the bloodstream, with subsequent pseudo-irreversible binding of GBR-hydroxy to DAT sites in the brain (Lewis et al., 1999). Although we have no direct pharmacokinetic evidence for this, the present autoradiographic data show that GBR-decanoate produces a significant decrease in ex vivo DAT binding (Fig. 7). The reduction in [125I]RTI-55 binding is DAT-specific because GBR-decanoate does not alter SERT binding (data not shown). Moreover, the decrease in [125I]RTI-55-labeled DAT binding parallels the neurochemical effects of GBR-decanoate, suggesting a correlating relationship between these variables. Kunko et al. (1997) observed that subchronic infusion of GBR12909 in rats (30 mg/kg/day for 7 days), which mimics the administration GBR-decanoate shown herein, dramatically reduces the $B_{max}$ for DAT binding in the striatum and nucleus accumbens. This effect is persistent, lasting several days after the cessation of GBR12909 infusion. We believe that subchronic infusion of GBR12909, or by analogy administration of GBR-decanoate, results in long-term reductions in DAT binding due to persistent occupation of transporter sites by the treatment drugs. Alternatively, it is possible that GBR analogs might diminish DAT binding by triggering internalization and proteolysis of DAT proteins (Daniels and Amara, 1999; Melikian and Buckley, 1999). Further experiments will be required to determine the precise molecular mechanism whereby GBR analogs decrease DAT binding.

In summary, the data presented herein show that GBR-decanoate produces long-lasting elevations in baseline extracellular DA and profound reductions in methamphetamine-evoked DA release. These effects are likely due to persistent occupation of DAT sites by GBR-hydroxy. A key question to be answered is the degree of DAT occupancy by GBR-hydroxy is necessary to attenuate methamphetamine-induced DA release. Studies using positron emission tomography in baboons indicate that doses of GBR12909 producing DAT occupancy levels of 30% reduce amphetamine-induced DA release by 75% (Villemagne et al., 1999). In humans, a 100-mg dose of GBR12909 occupies 30 to 40% of DAT sites in the caudate nucleus (Wong et al., 1999) and this dose of drug does not elicit stimulant-like subjective effects (So et al., 1997). Similar levels of DAT occupancy were obtained in the caudate nucleus (Wong et al., 1999) and this dose of drug does not elicit stimulant-like subjective effects (So et al., 1997). Thus, it seems feasible that daily oral administration of GBR12909, or periodic injections of GBR-decanoate, could significantly antagonize dopaminergic effects of methamphetamine. Clinical trials with these medications will be needed to determine the efficacy of such intervention.

References