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Cocaine and GBR12909 Produce Equivalent Motoric Responses at Different Occupancy of the Dopamine Transporter

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ROTHMAN, R. B., N. GREIG, A. KIM, B. R. DE COSTA, K. C. RICE, F. I. CARROLL AND A. PERT. *Cocaine and GBR12909 produce equivalent motoric responses at different occupancy of the dopamine transporter.* PHARMACOL BIOCHEM BEHAV 43(4) 1135-1142, 1992.—The *motoric*-stimulating effect of dopamine (DA) reuptake blockers is thought to result from the increase in synaptic dopamine levels, which occurs as a consequence of blockade of DA reuptake. The present study tested measured occupancy of the DA transporter in vivo produced by behaviorally equivalent doses of the DA reuptake blockers GBR12909 (20 mg/kg), cocaine (20 mg/kg),WIN35-065-2 (1 mg/kg), and nomifensine (5 mg/kg). Two methods were used to measure in vivo occupancy of the DA transporter: a) an ex vivo method, in which the ability of whole brain supernatants, prepared from rats administered the test drugs, were tested for their ability to inhibit the reuptake of [^{3}H]DA by striatal synaptosomes; and b) an in vivo binding assay using [^{3}H]*N*-[1-(2-benzo(*b*)thiophenyl)cyclohexyl]piperidine ([^{3}H]BTCP) to label the striatal DA transporter in vivo. Considerable data support the notion that this measurement is predictive of transporter occupancy in the nucleus accumbens. Similar results were obtained with both methods: The order of potency for apparent transporter occupancy was GBR12909 >> nomifensine > WIN35-065-2 = cocaine. These data indicate that it takes greater occupancy of the DA transporter by GBR12909 to produce behavioral effects equivalent to those produced by cocaine at lower transporter occupancy. The data of the present study suggest, therefore, that studies relating the effects of DA reuptake inhibitors on DA-mediated motoric behaviors to DA transporter occupancy might facilitate the identification of novel compounds potentially useful for the pharmacotherapy of cocaine abuse.

GBR12909 Nomifensine Cocaine Dopamine Locomotor activity

PREVIOUS studies from our laboratory (27) showed that administration of the high affinity dopamine (DA) reuptake blocker 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-[3-phenylpropyl]piperazine (GBR12909) (25 mg/kg, IP) produced small, long-lasting, and, at times, statistically insignificant increases in the levels of extracellular dopamine (ECDA) in the striatum of anesthetized rats. Despite this small effect, GBR12909 attenuated the ability of cocaine, administered through the microdialysis probe, to increase ECDA. This occurred when the effect of GBR12909 was calculated relative to the predrug baseline, as well as when the effect of GBR12909 was calculated relative to the baseline that existed prior to administration of cocaine (peak effect of cocaine − precocaine baseline). Related ex vivo ligand binding studies showed that at a dose of 25 mg/kg, GBR12909 was apparently persistently bound to about 50% of striatal DA transporters.

This pattern of having little effect on its own, yet blocking the effect of cocaine, suggests that when the dependent measurement is defined as being ECDA GBR12909 acts as an apparent partial agonist (27). It should be noted, however, that the phrase "partial agonist" does not imply a specific mechanism for the partial agonism. For example, a partial agonist can be defined as a drug that, when fully occupying a receptor, does not fully activate the effector system or the second messenger system associated with that receptor. In contrast to this "purist" definition, a partial agonist can be more traditionally defined on the basis of a pharmacological end point, usually many steps removed from the receptor-

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effector interaction. For example, nalorphine is commonly called a partial agonist, relative to morphine, because it is less efficacious as an analgesic and also blocks morphine-induced analgesia. The underlying mechanism of nalorphine's partial agonism results from the fact that it is both a κ-agonist and a μ-antagonist. Because κ-receptor-mediated analgesia is less effective than μ-receptor-mediated analgesia, nalorphine is less effective as an analgesic. Because nalorphine is a μ-antagonist, it will block morphine-induced analgesia. Thus, although nalorphine is a partial agonist in the traditional sense it is not a partial agonist according to the purist definition of partial agonism. As noted above, this is why we refer to GBR12909 as an apparent partial agonist. Clearly, additional studies will be needed to determine the mechanism of the apparent partial agonism of GBR12909.

The present study was undertaken to address the related question that GBR12909 is less efficacious at increasing DA-mediated motoric behaviors than is cocaine. The term "efficacy" refers to drug response as a function of receptor occupancy. For example, agent A is less efficacious than agent B if A produces the same response as B but at higher receptor occupancy. Similarly, agent A is more efficacious than agent B if A produces the same response as B but at lower receptor occupancy.

Whereas the effects of DA reuptake blockers on general motoric activity is thought to result from the increase in synaptic DA levels that occurs as a consequence of blockade of DA reuptake in the nucleus accumbens, stereotypy is thought to result from blockade of DA reuptake in the striatum (10,14–16). To test the hypothesis that GBR12909 and cocaine are equiefficacious in regard to stimulating DA-mediated motoric behaviors, the authors formulated the following prediction: Doses of GBR12909 and cocaine that produce the same degree of motoric stimulation should do so at identical occupancy of the DA transporter in vivo. The present study therefore determined the occupancy of the DA transporter in vivo produced by behaviorally equivalent doses of the DA reuptake blockers GBR12909, cocaine, WIN35-065-2, and nomifensine.

METHOD

Behavioral Measurements

Subjects. Male Sprague-Dawley rats weighing 250–275 g were housed 10 per cage and maintained on a light–dark cycle (light on 0700–1900 h) with food and water available ad lib. All animals were adapted to vivarium conditions for at least 1 week before experimentation. Testing was always performed between 1000–1700 h.

Behavioral Testing. Locomotor activity was assessed in photocell activity monitors (Omnitech Electronics, Inc., Columbus, OH), which were constructed of clear Plexiglas (30.5 × 42 cm × 42 cm). The activity chambers were enclosed in a sound-attenuating compartment equipped with a 15-W fluorescent light, a ventilating fan that also provided masking noise, and a one-way mirror (21 × 21 cm) mounted in the door to allow visual observation of animals during testing. A series of 16 equally spaced infrared photocell detectors were located along two adjacent walls of the chamber 4 cm from the floor surface. Interruptions of the infrared light sources by animals were recorded and stored by an IBM AT computer in 10-min intervals.

Stereotypy ratings were made in accordance with a rating scale modified from one devised by Kelly and Iversen (15). On this scale, behavioral responses by rodents are grouped into six distinct categories: 0 = alseep or stationary; 1 = active; 2 = predominantly active with bursts of stereotyped sniffing or rearing; 3 = stereotyped activity predominantly sniffing and rearing over a large area of the cage; 4 = stereotyped behavior maintained in one location; 5 = stereotyped behavior in one location with bursts of grooming and licking; 6 = continual grooming or licking of cage or cage contents.

Preliminary studies were conducted to determine behaviorally equivalent doses of the various uptake blockers. Rats were adapted to the locomotor chambers for 30 min and then injected IP with various doses of cocaine, GBR12909, nomifensine, and WIN35,065-2. All drugs were dissolved in solution consisting of 75% normal saline and 25% propylene glycol. Rats were allowed to remain in the locomotor chambers for an additional 60 min following injections. The results from these studies indicated that 2 mg/kg cocaine, 5 mg/kg nomifensine, 1 mg/kg WIN35,065-2, and 20 mg/kg GBR12909 produced equivalent increases in locomotor output 20–30 min following injection (Fig. 1). The behavioral effects appeared to be asymptomatic at this time interval for the doses in question.

Naive rats were again adapted to the apparatus as described above and then injected IP with either vehicle or the various drugs at the dosages indicated above. Horizontal locomotor activity was recorded in 10-min intervals and stereotypy was rated at 20 and 30 min following injections. At the end of 30 min, animals were decapitated and their brains rapidly removed and frozen in isopentane (−20°C). The frozen brains were stored at −70°C until needed.

Receptor Occupancy Measurements

Ex vivo administration of drugs. Frozen brains from the behavioral studies described above were later homogenized in 10 ml/g wet wt ice-cold 10 mM Tris-HCl, pH 7.4, and centrifuged at 30,000 × g for 20 min. Serial dilutions of the supernatants were then assayed for their ability to inhibit the specific reuptake of [^{3}H]DA into striatal synaptosomes (see below). This assay therefore measures the amount of reuptake blocker in whole-brain supernatants using the striatal [^{3}H]DA uptake system as a bioassay. The results were interpreted as follows: The more potent the supernatant, the higher the occupation of the DA transporter.

In vivo binding of [^{3}H]BTCP. [^{3}H]*N*-[1-(2-Benzo(*b*)thiophenyl)cyclohexyl]piperidine ([^{3}H]BRCP) has been shown to label the DA receptor complex in vivo (20,21). In the present study, at time 0 rats received IP injections of the reuptake blockers. At time 15 min, rats received IV injections, via the jugular vein, of [^{3}H]BTCP (100 μCi/kg, SA = 29.8 Ci/mMl). This dose (about 1.6 μg/kg is too low to produce behavioral effects (17). For determination of nonspecific binding, some rats received in addition 20 mg/kg BTCP (IV). At time 30 min, rats were sacrificed by decapitation and the caudates were dissected out and stored frozen at −70°C. Only gross behavioral measurements were made with these rats. The caudates were later homogenized with a polytron, while still frozen, in ice-cold 55.2 mM sodium phosphate buffer at pH 7.4. The homogenates were centrifuged at 30,000 × *g* for 10 min. The pellets were resuspended with 10 ml ice-cold buffer, and 1-ml aliquots were filtered (nine samples per homogenate) over Whatman GF/B filters (Whatman, Clifton, NJ), which were presoaked in 2% polyethylenimine. Control studies showed that specific [^{3}H]BTCP binding peaked at 15 min and that there was no detectable dissociation of prebound [^{3}HT]B-

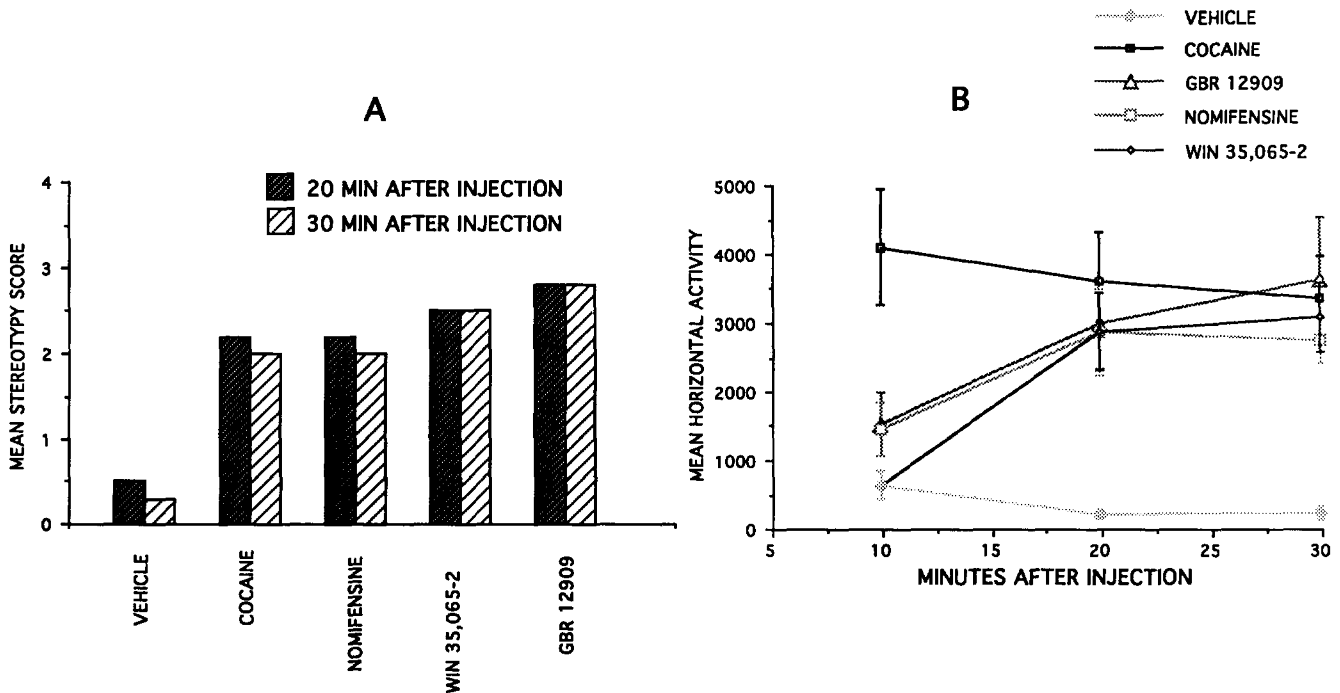

FIG. 1. Rats received IP injections of cocaine (20 mg/kg), nomifensine (5 mg/kg), WIN35,065-2 (1 mg/kg), and GBR12909 (20 mg/kg). Statistically indistinguishable effects on stereotypy (A) and locomotor activity (B) were observed (nonparametric Kruskal-Wallis one-way ANOVA; see text) (n = 5-6 rats per group).

TCP following the homogenization of the caudates. This method measures the amount of [^{3}HT]BTCP that is prebound to the transporter site in vivo. The tritium retained on the filters was counted, after an overnight extraction into 10 ml Beckman Redisolv (Beckman Instruments, Fullerton, CA) at 44% efficiency in a Micromedic Taurus Liquid scintillation counter.

[^{3}H]DA Reuptake

A crude preparation of striatal synaptosomes was prepared by homogenization, using a glass homogenizer with Teflon pestle, of freshly dissected rat caudate nuclei in ice-cold 10% sucrose (4.1 ml/caudate). Following centrifugation at 1,000 × g for 10 min, the supernatant was retained and kept on ice. Incubations were initiated by adding 0.1 ml of synaptosomes to 12 × 75-mm polystyrene test tubes prefilled with 10 nM [^{3}H]DA, which was delivered in 0.75 ml of a Krebs-phosphate buffer, containing 50 μM pargyline and 1 mg/ml ascorbic acid, pH 7.4 (buffer), and test drugs in 0.150 ml of buffer. Incubations were terminated after a 15-min incubation at 25°C by filtration over Whatman BF/B filters, which were washed twice with 4 ml 0.9% NaCl buffered with 10 mM Tris-HCl buffer, pH 7.4. Nonspecific reuptake was measured using 1 μM GBR12909. Control studies indicated that specific reuptake was a) linear with time up to 30 min and b) directly proportional to protein in the protein range used here. The Krebs-phosphate buffer contained 154.5 mM NaCl, 2.9 mM KCl, 1.1 mM $CaCl_2$, 0.83 mM $MgCl_2$, and 5 mM glucose. The tritium retained on the filters was counted, after an overnight extraction into 10 ml Beckman Redisolv, at 44% efficiency in a Micromedic Taurus Liquid scintillation counter.

Chemicals

[^{3}H]DA (SA = 20 Ci/mM) was purchased from Dupont New England Nuclear Corp. (Newton, MA) [^{3}H]BTCP (SA = 29.8 Ci/mM), BTCP, and GBR12909 were prepared in the Laboratory or Medicinal Chemistry, NIDDK (Bethesda, MD), as described (7,30). WIN35,065-2 was synthesized at Research Triangle Institute (Research Triangle Park, NC). Nomifensine was kindly provided by Dr. Paul Berger, Neuroscience Branch, NIMH (Bethesda, MD). Cocaine was provided by the NIDA (Baltimore, MD).

RESULTS

Ex Vivo Administration of Drugs

Cocaine (20 mg/kg), nomifensine (5 mg/kg), WIN35,065-2 (1 mg/kg), and GBR12909 (20 mg/kg) produced statistically indistinguishable effects on stereotypy (Fig. 1A) and locomotor activity (Fig. 1B). The brains from these animals were collected and stored at −70°C as described in the Method section.

The nonparametric Kruskal-Wallis one-way analysis of variance (ANOVA) did not reveal a statistically significant difference among the stereotypy scores of the various drug treatment groups at either 20 or 30 min after injection ($p >$ 0.05). The Mann-Whitney U-test indicated that all of the drug treatment groups were significantly different ($p < 0.05$) from the vehicle group at both time points.

A repeated-measures ANOVA revealed a significant drug treatment effect, $F(4, 23) = 6.55$, $p < 0.01$), and a significant effect of time, $F(2, 46) = 7.50$, $p < 0.01$, as well as a significant drug × time interaction, $F(8, 46) = 6.17$, $p <$

0.01. There was a major difference between the time course of cocaine and the other three uptake blockers that contributed to the significant time and time × drug interaction (Fig. 1B). By the third sampling period, however, there was little apparent difference in motoric output between the four drug treatment groups. A one-way ANOVA of locomotor activity during this interval revealed a significant treatment effect attributed solely to difference in locomotor behavior between the vehicle group and the four drug groups that did not differ among themselves [$F(4, 23) = 6.64$, $p < 0.01$ for comparisons among treatment groups during the third sampling interval]. The Fisher PLSD test revealed that the vehicle group differed significantly ($p < 0.05$) from the four drug treatment groups during this interval while none of the drug treatment groups differed among themselves ($p > 0.05$).

As reported in Fig. 2, supernatants prepared from brains produced a dose-dependent inhibition of [^{3}H]DA reuptake. Supernatant prepared from the brains of saline-injected rats were without effect until the 125-μl dose and above, at which point moderate inhibition was observed (28% with 125 μl, 51.5% with 250 μl, 65.6% with 500 μl). Therefore, data were calculated as a percent of the saline supernatants. GBR12909-2 supernatant was by far the most potent (Fig. 2).

As reported in Table 1, whereas GBR12909 supernatant produced statistically significant inhibition of [^{3}H]DA reuptake at concentrations as low as 6.25 μl the other supernatants did not begin to produce inhibition until a dose of 125 λ. Control experiments indicated that incubation of the reuptake agents with supernatant prepared from control animals for 1 h at 25°C did not increase their inhibitory activity (data not shown), demonstrating that differences in the inhibitory potencies of the supernatants is not due to metabolism of the reuptake blockers by the supernatant. The order of potency for apparent transporter occupancy was GBR12909 >> nomifensine > WIN35-065-2 = cocaine. To validate the [^{3}H]DA reuptake assay, we determined the IC_{50} values of the test agents in vitro. The results were: GBR12909 (9.9 nM), cocaine (728 nM), WIN35,065-2 (168 nM), and nomifensine (149 nM).

TABLE 1

STATISTICAL SIGNIFICANCE TABLE

Saline vs.	Supernatant (μl) 3.1	6.2	12.5	25	50	100	125	250	500
Cocaine	NS	NS	NS	NS	NS	NS	NS	S	S
Nomifensine	NS	NS	NS	NS	NS	NS	S	S	S
WIN35,065-2	NS	NS	NS	NS	NS	NS	S	S	S
GBR12909	NS	S	S	S	S	S	S	S	S

Statistical significance was determined using ANOVA with the posthoc Scheffe's F-test. NS, not significant; S, $p < 0.05$.

In Vivo Binding Studies

As reported in Fig. 3, all four reuptake inhibitors produced significant decreases in the specific binding of [^{3}HT]BTCP to the DA transporter. GBR12909 was by far the most potent in that it completely inhibited [^{3}HT]BTCP binding. The percent occupancy of the DA transporter was calculated as the percent

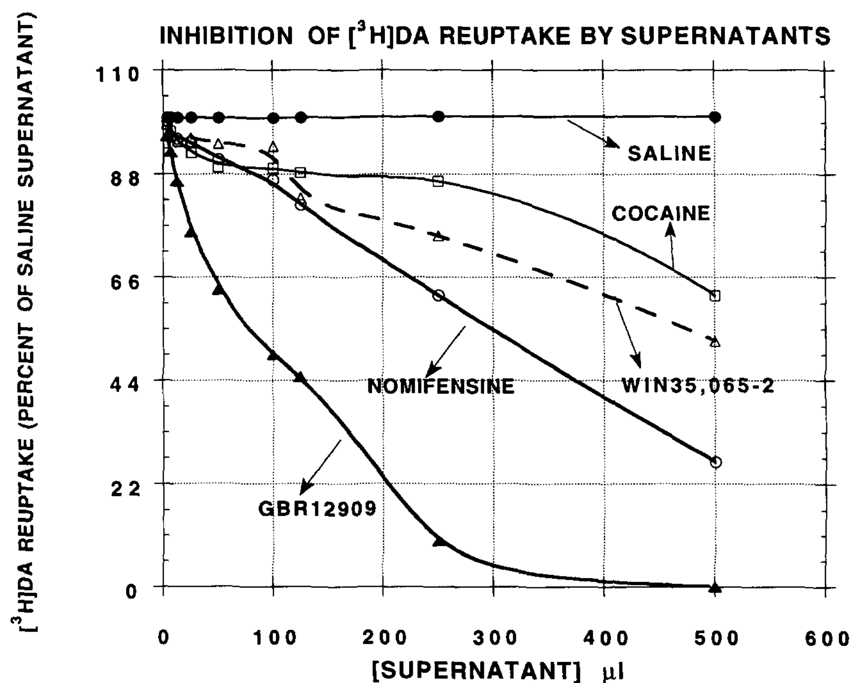

FIG. 2. Aliquots of brain supernatants prepared from rats injected with cocaine (20 mg/kg), nomifensine (5 mg/kg), WIN35,065-2 (1 mg/kg), and GBR12909 (20 mg/kg) were assayed for their ability to inhibit [^{3}H]DA reuptake into striatal synaptosomes. SD values were less than 10% of the mean (n = 5-6 rats per group). The [^{3}H]DA reuptake assay took place for 15 min at 25°C.

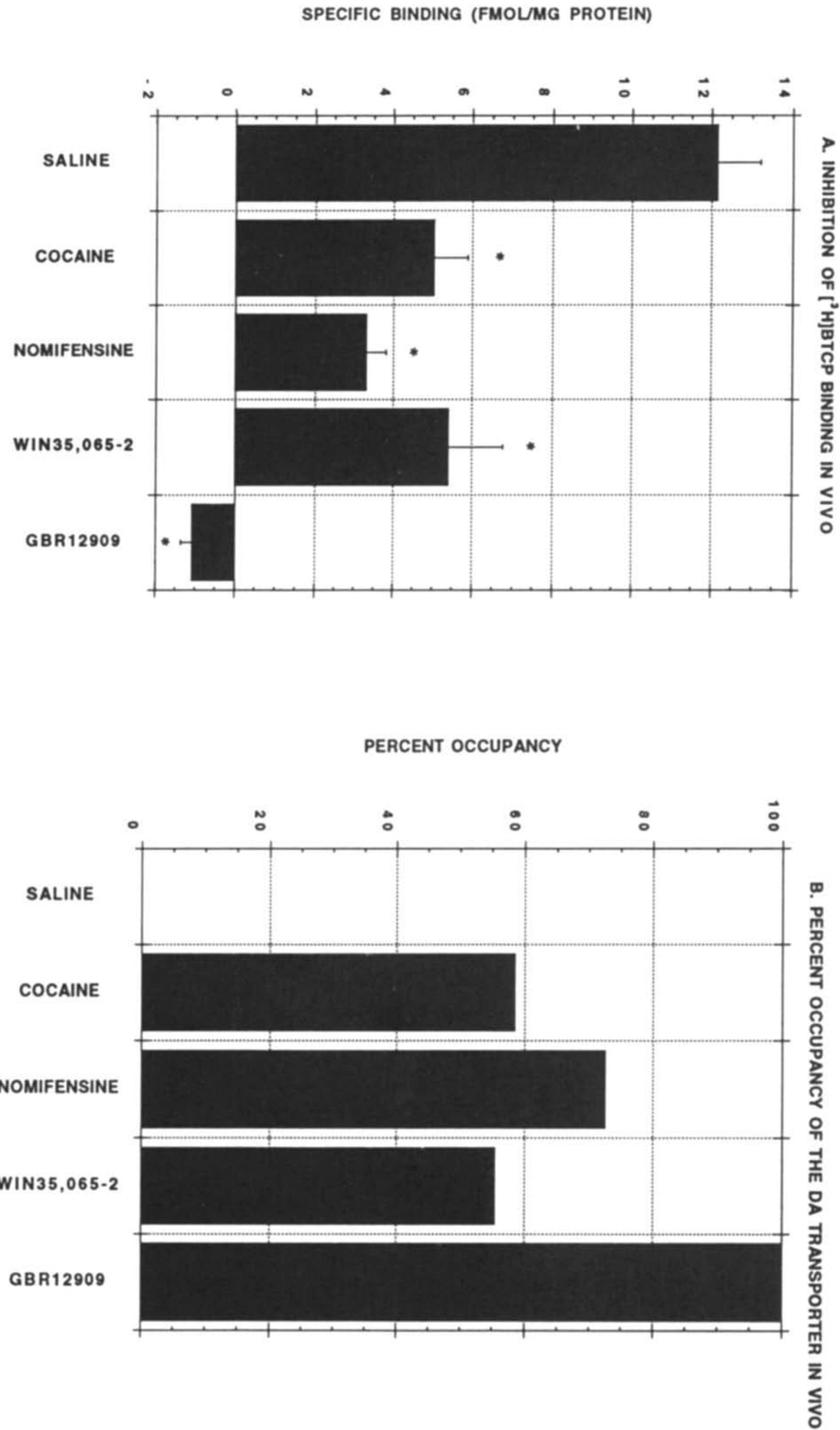

FIG. 3. In vivo binding studies were carried out as described in the Method section. (A). Specific binding. (B). Data are recalculated as percent occupancy. $*p < 0.05$ when compared to saline (ANOVA, posthoc Scheffe's F-test, n = 5–6 rats per group).

inhibition. These data are shown in Fig. 3B. The order of potency of transporter occupancy was: GBR12909 >> nomifensine > WIN35-065-2 = cocaine.

CONCLUSIONS

The major goal of this study was to relate the DA-mediated motoric effects of GBR12909 and other DA reuptake blockers to their occupancy of the DA transporter in vivo. To do this, we first determined doses of these agents that produced statistically indistinguishable effects on locomotor activity and stereotypy, thought to reflect dopaminergic activity in the nucleus accumbens and striatum, respectively. As noted below, although the transporter occupancy measurements were made in the striatum considerable data support the notion that this measurement is predictive of receptor occupancy in the nucleus accumbens. The next stage of the study required measuring the occupancy of the DA transporter at these behaviorally equivalent doses.

Because no method of measuring in vivo receptor occupancy is without some limitations, two methods were used. In the ex vivo approach, brains are collected at the end of the 30-min measurement period in the locomotor cage and supernatants are prepared. These are subsequently assayed for inhibitory activity. In the simplest scenario, two agents producing the same effect, at the same degree of transporter occupancy, would be expected to have equipotent supernatants. Similarly, increased inhibition of [^{3}H]DA reuptake in vitro by one supernatant relative to another can be taken as evidence for increased transporter occupancy in vivo. This assay method measures whole-brain levels of reuptake inhibitor using striatal uptake of [^{3}H]DA as the bioassay. An assumption of this assay is that whole brain levels accurately reflect drug levels in striatum and nucleus accumbens and that the striatal DA transporter does not differ from the DA transporter of the nucleus accumbens. These assumptions are reasonable and supported by the data (see below).

Another reasonable assumption of the ex vivo method is that the IC_{50} values of the agents used in this study for inhibition of DA reuptake in vivo are highly correlated with their IC_{50} values for inhibition of [^{3}H]DA reuptake in vitro (1). In addition, this method cannot control for redistribution of drug, which may or may not occur during homogenization of the tissue. Advantages of this method include the high signal-to-noise ratio provided by the robust [^{3}H]DA reuptake assay and the fact that total inhibitory activity is measured (intact drug plus active metabolites).

An in vivo binding assay provides, in principle, a more straightforward determination of transporter occupancy in vivo. The most common approach for conducting in vivo binding assays calls for administration of the [^{3}H]ligand, a washout period during which the nonspecific binding is eliminated from the brain, and then measurement of total tissue tritium (25). The difficulty posed by this method for measuring in vivo occupancy is meshing the complex pharmacokinetics of the in vivo binding process with the pharmacokinetics of the test drug. For example, the optimal signal-to-noise ratio might occur with a 120-min washout period whereas the test drug might achieve peak brain levels in 15 min.

The in vivo binding method used here, similar to the methods used by Perry et al. (24) and Maurice et al. (20), minimizes this problem. The animal is sacrificed at an appropriate time after administration of the [^{3}H]ligand and the brain tissue is collected, homogenized, and filtered, much as is done in an in vitro binding assay. [^{3}H]ligand that is not tightly bound to the receptor is removed by the rapid filtration procedure. In the present study, control studies indicated that peak specific binding was achieved in 15 min, about how long it takes for peak behavioral effects to occur.

The in vivo binding protocol used here called for administration of [^{3}H]BTCP 15 min after administration of test drugs. Because animals were sacrificed 30 min after administration of the test drugs, the measurement of in vivo binding was taken at the time of peak [^{3}HT]BTCP binding but at less than the time of peak binding for the test drugs. More importantly, the measurement was taken at the time of behaviorally equivalent responses. Another advantage of this time line is that the in vivo binding measurement was taken at the same time as in the ex vivo experiments, where rats were sacrificed at the end of the 30-min test session. The relatively low signal-to-noise ratio of the in vivo binding assay (about 50% specific binding) also dictated that rats be sacrificed at the time of peak specific binding.

Despite the limitations of each of these methods for estimating in vivo occupancy of the DA transporter, both methods yielded the same result. The simplest interpretation of the results is that considerably more DA transporters have to be occupied by GBR12909 than by cocaine to produce the same degree of motoric activation. Although the data seemed to indicate that nomifensine had higher transporter occupancy than cocaine, the differences were small. Given the high variance of the behavioral measures, and the low signal-to-noise ratio of the in vivo binding assay, it is likely that these small differences are not significant. Clearly, methodological advances will have to be made to be able to reliably measure small differences in transporter occupancy as it relates to a behavioral measure.

Another important methodological issue is that the receptor occupancy measurements determined with [^{3}HT]BTCP were done in caudate whereas the behavioral end point of greatest interest, locomotor activity, reflects transporter occupancy in the nucleus accumbens. Clearly, the assumptions of this approach are that the distribution of drug to the two brain regions are correlated and that the DA transporters in the two brain regions do not differ. Regarding the first assumption, there is no reason to expect that the drug distribution to the striatum and nucleus accumbens differ. Regarding the second assumption, although the striatal and nucleus accumbens DA transporters differ slightly in molecular weight, probably due to differences in glycosylation (18,19), several studies have shown no significant differences between the binding and functional properties of the DA transporter in these two brain regions (2,3,11,12). The similar pharmacokinetics of the test drugs and their similar pharmacodynamic interaction with the DA transporter in both brain regions strongly suggest that receptor occupancy is the same in both brain regions. These observations therefore support the use of the striatal DA transporter, as is commonly done in most in vitro studies, as a model of the nucleus accumbens DA transporter.

It is interesting to note that the variances of the ex vivo and in vivo binding data were all considerably less than what was observed for the behavioral measures, indicating that the variance of the behavioral measures does not result from individual differences in the pharmacokinetics of the test drugs. The variances of behavioral measures obtained using genetically inbred strains of mice are apparently less than the variances usually obtained using outbred strains of rats (29). Thus, it is possible that the use of genetically inbred strains of mice, combined with a higher specific activity ligand for the DA transporter, such as [^{125}I]RTI-55 (4), might together pro-

vide an assay system with enough precision to detect small differences in transporter occupancy.

The finding that at behaviorally equivalent doses GBR12909 occupies considerably more transporters than does cocaine supports the hypothesis that GBR12909 is less efficacious than cocaine in increasing DA-mediated motoric behaviors. There are several possible mechanisms that might explain this finding.

One possible mechanism is an action of GBR12909 at sites/receptors other than the DA transporter that inhibits its motoric effects. Thus, higher transporter occupancy might be required to produce the same motoric activity as that produced by cocaine. For example, we (28) and others (23) have suggested that ligands with high affinity for the σ-binding site might negatively modulate effects of cocaine. In this regard, GBR12909 has high affinity for the σ-binding site (5). The present study does not directly address the σ-hypothesis. However, we feel that it is unlikely because GBR12935, which has much lower affinity for the σ-site than does GBR12909 (K_i about 50 nM), is equipotent with GBR12909 as a locomotor stimulant (unpublished data) yet produces a similarly high degree of transporter occupancy in vivo (27). Related possible mechanisms include GBR12909-induced decreases in the firing rate of DAergic nerves and decreased synthesis of DA.

In this regard, it should be noted that a dose of 25 mg/kg GBR12909 results in a brain concentration of about 14 μM (27). This concentration would certainly be high enough to interact at other sites where GBR12909 has moderate affinity, such as the sodium channel and D_2 receptor (1). However, GBR12909 is lipophilic and the majority of the drug is almost certainly dissolved in brain lipid. In support of this, Menacherry and Justice (22) determined that a 100-mg/kg IP dose of GBR12909 in the brain extracellular fluid, a hydrophilic compartment. In addition, if the effective concentration of GBR12909 available for binding to the DA transporter were actually 14 μM then all the DA transporters would be completely occupied and cocaine should be totally ineffective in elevating ECDA. However, as reported in our microdialysis study (27), cocaine quite effectively increased ECDA in rats pretreated with 25 mg/kg GBR12909, demonstrating that all DA transporters were not blocked. Nevertheless, an "other action" hypothesis can never be conclusively ruled out because a drug can always have effects the significance of which is either not recognized or appreciated.

Another possible mechanism is that cocaine could be acting at sites/receptors other than the DA transporter to increase its locomotor activating effects. For example, cocaine blocks the reuptake of 5-hydroxytryptamine (5-HT), which, acting through 5-HT_3 receptors, might increase DA release (8,9,13). This hypothesis predicts that a selective serotonin reuptake blocker should potentiate the locomotor-stimulating effects of a selective DA reuptake blocker. This hypothesis should be addressed in future studies.

Another possible mechanism is that cocaine and GBR12909 have different intrinsic activity as inhibitors of DA reuptake in vivo, that is, higher transporter occupancy by GBR12909 produces the same degree of transporter inhibition as lower transporter occupancy by cocaine. Because we did not directly measure DA reuptake or ECDA levels in this study, the reasonableness of considering such a mechanism rests entirely on the assumption that: a) The motoric activity produced by DA reuptake inhibitors results from increased synaptic levels of DA, b) two DA reuptake blockers that produce equal motoric activity are producing equal levels of synaptic DA, and c) the DA reuptake inhibitors do not have some other action that either attenuates or facilitates their locomotor-stimulating effects (see above). Clearly, the present study was not designed to rigorously test this mechanism: To do so would require measuring inhibition of DA and binding to the transporter at the same time, under identical conditions, and using selective agents that do not have other actions.

Regardless of which mechanism might explain the observation that behaviorally equivalent doses of GBR12909 occupy considerably more DA transporters than does cocaine, such a drug could turn out to be therapeutically useful as either substitution therapy for, or an antagonist of, cocaine. For example, such a drug could completely occupy the DA transporter and thereby block the reinforcing effects of cocaine (26), yet have its own dopaminergic effects attenuated by its other action. The data of the present study suggest, therefore, that studies relating the effects of DA reuptake inhibitors on DA-mediated motoric activity to DA transporter occupancy might facilitate the identification of novel compounds potentially useful for the pharmacotherapy of cocaine abuse.

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