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Negative interaction of dopamine D2 receptor antagonists and GBR 12909 and GBR 12935 dopamine uptake inhibitors in the nucleus accumbens

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Abstract

The objective of this study was to examine the interaction of dopamine D2 receptor antagonists and dopamine uptake inhibitors on the regulation of extracellular dopamine release in the nucleus accumbens of Wistar rats employing in vivo microdialysis and in vitro dopamine uptake studies. Application of the D2 receptor antagonists raclopride (100 μm) or sulpiride (100 μm) alone through the microdialysis probe in the nucleus accumbens for 60 min increased the extracellular levels of dopamine in the nucleus accumbens to 150% and 200% of basal, respectively. Perfusion of the nucleus accumbens for 60 min with the dopamine uptake inhibitors, 1-[2-[bis(4-Fluorophenyl)methoxy]ethyl]-4-[3-phenylpropyl]piperazine dihydrochloride (GBR 12909; 100 μm) or 1-[2-(Diphenylmethoxy)ethyl]-4-(3-phenylpropyl)-piperazine dihydrochloride (GBR 12935; 100 μm) alone, increased the extracellular levels of dopamine in the nucleus accumbens to 400% and 350% of basal, respectively. Co-perfusion of 100 μM GBR 12909 or GBR 12935 with either 100 μM sulpiride or raclopride produced a significant reduction in the GBR 12909 or GBR 12935 induced increase in the extracellular levels of dopamine to basal levels. In vitro, GBR 12909 (1–9 nM) dose-dependently inhibited active uptake of [^{3}H]dopamine in homogenates of the nucleus accumbens. Addition of 100 μm sulpiride had little effect on GBR 12909 inhibition of [^{3}H] dopamine uptake, suggesting that dopamine D2 receptor antagonists are not blocking the actions of the GBR-type dopamine uptake inhibitors at the dopamine transporter. Overall, the data suggest that complex interactions occur in vivo between D2 antagonists and GBR-type dopamine uptake inhibitors, which negate their effects on elevating the extracellular levels of dopamine in the nucleus accumbens. © 2001 Published by Elsevier Science B.V.

Keywords: Nucleus accumbens; Dopamine uptake; GBR 12909; GBR 12935; Sulpiride; Raclopride; Microdialysis, in vivo

1. Introduction

Midbrain dopamine neurons originate in the ventral tegmental area and innervate nucleus accumbens have been implicated in mediating the actions of many drugs of abuse (Koob, 1992; Di Chiara, 1995; Wise, 1996; Spanagel and Weiss, 1999). A growing body of evidence has confirmed the presence of various dopamine receptors subtypes in the nucleus accumbens, including the dopamine D2 receptor (White and Wang, 1986; Bardo and Hammer, 1991; Kalivas and Duffy, 1991; Shetreat et al., 1996; Westerink et al., 1996). Dopamine D2 receptors have been identified as post-synaptic dopamine receptors in apposition to dopamine terminals within the nucleus accumbens, and presynaptic dopamine autoreceptors located on dopamine terminals (see Missale et al., 1998, for review). Furthermore, as in the striatum (Imperato and Di Chiara, 1988; Westerink and De Vries, 1989) and prefrontal cortex (Santiago et al., 1993), local application of the dopamine D2 receptor antagonist sulpiride alone through the microdialysis probe elevated the extracellular levels of dopamine in the nucleus accumbens (Rahman and McBride, 2000).

Selective dopamine uptake inhibitors 1-[2-[bis(4-Fluorophenyl)methoxy]ethyl]-4-[3-phenylpropyl]piperazine dihydrochloride (GBR 12909) or 1-[2-(Diphenylmethoxy)-ethyl]-4-(3-phenylpropyl)-piperazine dihydrochloride (GBR 12935) are known to enhance dopamine transmission in the target region(s), including the nucleus accumbens, by inhibiting reuptake into the nerve terminals (Andersen, 1989; Li et al., 1996; Nomikos et al., 1990;

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Reith et al., 1997). These agents inhibit the uptake of dopamine with IC_{50} values in the nanomolar range and are several-fold less potent as inhibitors of the uptake of norepinephrine and serotonin (Van der Zee et al., 1980; Heikkila and Manzino, 1984; Bonnet and Costentin, 1986; Andersen, 1987, 1989). Moreover, unlike other dopamine uptake inhibitors (Herdon et al., 1987), GBR-type compounds have a very low potency to induce the release of dopamine (Van der Zee et al., 1980; Heikkila and Manzino, 1984; Bonnet and Costentin, 1986). Therefore, GBR-type compounds may have neurochemical features distinct from conventional dopamine uptake blockers (Andersen, 1989).

GBR 12909 or GBR 12935 elevated the extracellular dopamine levels in the nucleus accumbens following local application through the microdialysis probe (Engleman et al., 2000; Li et al., 1996; Kohl et al, 1998; Rahman and McBride, 2000). Recently, while investigating the feedback regulation of mesolimbic somatodendritic dopamine release, we observed that co-perfusion of the dopamine D2 receptor antagonist sulpiride, with nomifensine (dopamine uptake inhibitor), exhibited a synergistic elevation of extracellular dopamine levels in the nucleus accumbens as a result of the combination of inhibiting reuptake of dopamine as well as blocking local and long-loop feedback inhibition (Rahman and McBride, 2000). However, a preliminary study undertaken at that time using GBR 12909 with sulpiride indicated that not only was there no similar synergistic interaction, but when both compounds were co-perfused there was no change in the extracellular levels of dopamine in the nucleus accumbens. These results suggested a unique interaction between the GBR-type dopamine uptake inhibitors and dopamine D2 receptor antagonists. Therefore, the present study was undertaken to examine the interaction of dopamine D2 receptor antagonists and GBR-type dopamine uptake inhibitors employing in vivo microdialysis and in vitro uptake assays. The hypothesis to be tested is that there are inhibitory interactions between dopamine D2 receptor antagonists and GBR-type dopamine uptake inhibitors in the nucleus accumbens.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (250–350 g; Harlan, Indianapolis, IN, USA) were used in this study. Rats were singly housed and maintained on a normal 12-h light/dark cycle (lights on 0700 h) in a constant temperature and humidity controlled animal facility with food and water ad libitum.

2.2. Drugs / chemicals

The following agents were used: (a) dopamine uptake inhibitors, 1-[2-[bis(4-Fluorophenyl)methoxy]ethyl]-4-[3-phenylpropyl]piperazine dihydrochloride (GBR 12909 · 2HCl) and 1-[2-(Diphenylmethoxy)ethyl]-4-(3-phenylpropyl)-piperazine dihydrochloride (GBR 12935 · 2HCl; (b) the D2 antagonist S(−)-Sulpiride and S(+)-Raclopride-L-tartrate from Research Biochemicals, Natick, MA, USA. All agents were dissolved in artificial cerebrospinal fluid (see below) and perfused through the microdialysis probe in the nucleus accumbens for in vivo microdialysis studies. 3-4-[ring-2,5,6-^{3}H]dihydroxyphenylethylamine was obtained from New England Nuclear (Boston, MA, USA) and used for the dopamine uptake studies.

2.3. Microdialysis

Rats were anaesthetized under 1–2% isoflurane and placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA); the skull was exposed and a small hole was drilled to insert a guide cannula. Rats were maintained on a 37°C heating pad throughout the course of surgery. A microdialysis guide cannula (18 gauge; Plastics One, Roanoke, VA, USA) was implanted in the nucleus accumbens according to the atlas of Paxinos and Watson (1986). The cannula was implanted at a 10° angle from the midline using the following coordinates relative to bregma with the incisor bar set at −3.3 mm: anterior–posterior +1.7 mm, lateral +2.4 mm, and dorsal/ventral −6.3 mm. The guide cannula was slowly (1 mm/min) inserted into position; three stainless steel screws were placed in the skull to secure the guides, and the guides were fixed in place with cranioplastic cement (Plastics One). One stainless steel dummy probe, cut to extend to the tip of the guide cannula, was inserted to maintain patency. Rats were allowed to recover for 5–6 days in their home cages following surgery, during which they were allowed free access to food and water. Animals used in these procedures were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care. All research protocols were approved by the Institutional Animal Care and Use Committee and are in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, NIH, and the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

The loop style probes were made as previously described (Rahman and McBride, 2000). The active area of the probe tip was 2 mm in length; the total length of the dialysis membrane was 4 mm. The outside diameter of the dialysis membrane was 220 μm. Representative probe placements in the nucleus accumbens are illustrated (Fig. 1). Within the nucleus accumbens, almost all of the probes perfused both the core and shell to varying degrees, with some placements mostly in the shell and some placements mostly in the core. A few probes did have a small portion of the active membrane in the striatum, and some probes had tips close to the olfactory tubercle. Therefore, it is

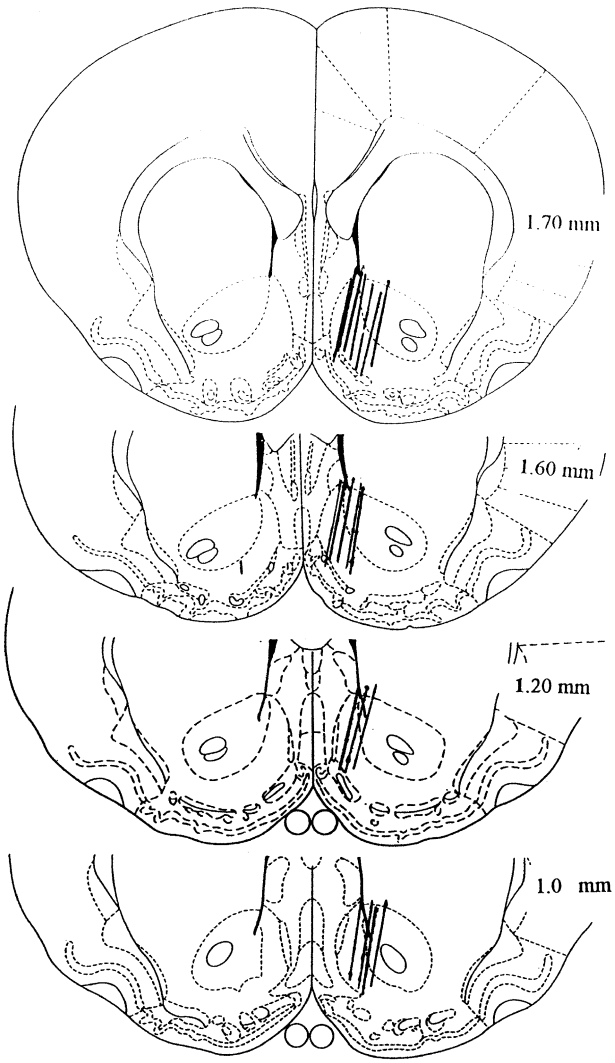

Fig. 1. Representative locations of microdialysis probe placements in the nucleus accumbens. Overlapping placements are not shown; therefore, the complete quantitative distribution of the placements is not indicated here. Numbers on the right indicate the distance (mm) from bregma (Paxinos and Watson, 1986). The line represents the 2-mm active area of the probe. Most probe placements were in the shell and shell plus core portions of the nucleus accumbens.

likely that the dopamine collected in the dialysis samples is mainly from the core and shell combined.

On post-surgery day 5, the rats were transferred to the Plexiglas chambers ($25.5 \times 44.5 \times 38.0$ cm, width $\times$ length $\times$ height), used during microdialysis, for daily handling and habituation to the chambers. On post-surgery day 6, the rats were briefly anaesthetized with isoflurane and the loop style probe was inserted through the guide and cemented into place. The following day (day 7 post-surgery), rats were placed in the Plexiglas chambers. Experiments were performed in freely moving animals. Food and water were not available during microdialysis. The inputs of the dialysis probes were connected to a syringe pump (Harvard Instruments, South Natick, MA, USA), which delivered artificial cerebrospinal fluid to the probe at a rate of 0.6 μl/min. The artificial cerebrospinal fluid (composition in mM: NaCl, 145; KCl, 2.7; $MgCl_2$, 1.0; $CaCl_2$, 1.2; pH adjusted to 7.4 ± 0.2 with 2 mM Na_2HPO_4) was filtered through a 0.2-μm sterile filter. The artificial cerebrospinal fluid was perfused for 60–90 min before any baseline samples were collected. Baseline samples were collected every 20 min for an additional 60 min before introducing any dopamine agent. Stable baseline values for the extracellular levels of dopamine in nucleus accumbens usually occurred within 60 min, as previously reported (Kohl et al., 1998). Samples were collected in 0.5-ml polyethylene tubes containing 3 μl of 0.05 N $HClO_4$ and were either analyzed directly or immediately frozen on dry ice and stored at -70°C until analysis. Frozen samples showed no sign of degradation for up to 1 month.

Dopamine uptake inhibitors GBR 12909 or 12935 were perfused through the probe for 60 min to determine the changes from basal levels of extracellular dopamine in the nucleus accumbens. Separate groups of rats were perfused with (a) an uptake inhibitor alone, (b) an antagonist alone, or (c) a combination of uptake inhibitor plus antagonist. Samples were collected prior to, during and 60 min after perfusion.

At the end of the experiment, 1% bromphenol blue solution was perfused through the probes to verify the placements. Rats were then overdosed with CO_2 and decapitated, and the brains removed. Brains were then stored at -70°C; frozen 40 μm coronal sections were prepared and stained with Cresyl violet dye for verification of the probe tips. Probe placements were evaluated according to the atlas of Paxinos and Watson (1986).

2.4. Sample analysis

Samples were analyzed by a microbore high-performance liquid chromatography with an electrochemical detection system as previously described (Rahman and McBride, 2000) to determine dopamine levels in each sample. Briefly, chromatography was performed using a model 2350 pump (ISCO, Lincoln, NE, USA) with a Bioanalytical Systems (BAS) SepStik microbore analytical column (1.0×100 mm column; 3 μm Spherisorb C18 stationary phase) connected to BAS custom injection valve and a Rheodyne 5.0-μl injection loop mounted in a Unijet model CC-6 cabinet (BAS, West Lafayette, IN, USA). A pulse damper (Scientific Systems, State College, PA, USA) and chromatography column (Waters Spherisorb 5 um ODS-2, 3×100 mm; Keystone Scientific, Bellefonte, PA) were installed in-line between the pump and injection valve to further reduce pump pulsations and to increase system back pressure, respectively. The mobile phase was composed of 100 mM sodium acetate, 0.5 mM EDTA, 5 mM sodium octanesulfonic acid, 10 mM NaCl and 6% acetonitrile; pH 4.0 adjusted with glacial acetic acid. The mobile phase was briefly bubbled with helium to de-

oxygenate it. The column was maintained at room temperature and the flow rate was 75 μl/min. Dopamine was detected with a BAS Unijet radial-flow detector cell with a 6-mm glassy carbon electrode (BAS) coupled to a model 400 detector via an external cell cable (EG & G Princeton Applied Research, Princeton, NJ). The applied potential was set at +450 mV with a sensitivity setting of 0.5 nA/V. The use of the Unijet reference electrode required an applied potential setting that was 100 mV less than the equivalent potential setting for a standard Ag/AgCl reference electrode. The output of the detector was sent to a Chrom Perfect (Justice Innovations, Palo Alto, CA) chromatography data analysis system. The lower limit of sensitivity for dopamine was approximately 0.2 fmol injected onto the column.

2.5. [^{3}H]dopamine uptake

The rats were killed by decapitation; the brains were removed and placed on a petri dish on ice. The nucleus accumbens was dissected bilaterally, weighed and homogenized in 100 volumes of 0.32 M sucrose. Uptake of dopamine was determined as previously described (Simon et al., 1997) with slight modification. Briefly, crude synaptosomes were incubated in 50 μl of Krebs–Ringer phosphate buffer (pH 7.4) containing 10 nM [^{3}H] dopamine (final specific activity 60 Ci/mmol). Following a 2-min incubation at 30°C, uptake was terminated by centrifugation at $6000 \times g$ for 15 min at 4°C. Supernatants were aspirated and the pellets were surface washed with 2 ml of ice cold saline. Pellets were dissolved in 0.5 ml of 0.1 M NaOH and radioactivity was determined by liquid scintillation spectrometry following the addition of 5 ml of EcoLite scintillation cocktail (ICN, Costa Mesa, CA, USA). Blank values for dopamine uptake were determined by incubating the samples in the presence of 0.5 μM GBR 12935. The Krebs–Ringer phosphate buffer (pH 7.4) had the following composition: 126 mM NaCl, 4.75 mM KCl, 1.3 mM $CaCl_2$, 15.8 mM Na_2HPO_4, 1.4 mM $MgCl_2$ and 10 mM dextrose. When drugs were present during the uptake assay, they were added from 50-fold concentrated stock solutions prepared with deionized H_2O. Protein concentration of the homogenate was determined by the method of Bradford (1976) using bovine serum albumin as the standard.

2.6. Data analysis

Dialysate values were not corrected for probe recovery efficiency, which is approximately 11% and in close agreement with published values (Perry and Fuller, 1992; Rahman and McBride, 2000). To minimize rat to rat variability, data for the individual experiments were normalized and expressed as percent change from baseline values. Percent baseline levels for each experiment were calculated as treatment/baseline × 100. The average concentration of three stable samples prior to perfusion with one of the dopamine agents (< 10% variation) was considered the baseline and was defined as 100%. Data were analyzed using the statistical program SPSS. Normalized data were analyzed by two-way analysis of variance (ANOVA) with repeated measures, followed by the least significant difference (LSD) test for multiple comparisons when appropriate. The comparisons of GBR 12909 or 12935 solutions with and without sulpiride or raclopride were planned during experimental design. The significance level was set at $P < 0.05$. The details of the statistical analysis are contained in the figure legends.

3. Results

3.1. Probe placements

Only data from animals that had probes correctly implanted in the nucleus accumbens were included in this study. Most (~85%) of the animals which had undergone surgery had probes implanted in the nucleus accumbens. The loop-style probe was oriented along the anteroposterior axis of the nucleus accumbens, and therefore a major portion of the region was perfused. Fig. 1 shows representative probe placements in the nucleus accumbens; it does not show overlapping probe placements and is not a quantitative representation of the distribution of probe placements. Within the nucleus accumbens, almost all of the probes perfused both the core and shell to varying degrees, with some placements mostly in the shell and some placements mostly in the core. A few probes had tips close to the olfactory tubercle. Because such a small portion of the active membrane is exposed to tissue outside the nucleus accumbens, it is likely that the dopamine collected in the dialysis samples is mainly from the core and shell combined.

3.2. Effects of dopamine receptor antagonists and GBR-type compounds on extracellular levels of dopamine

Local application of 100 μM sulpiride or raclopride, dopamine D2 receptor antagonists, through the microdialysis probe in the nucleus accumbens increased the extracellular levels of dopamine to 200% and 150% of basal, respectively (Fig. 2). Similarly, perfusion of GBR 12909, a selective dopamine uptake inhibitor, in the nucleus accumbens significantly increased the extracellular levels of dopamine to approximately 400% of basal. However, coperfusion of 100 μm sulpiride or raclopride with GBR 12909 did not significantly alter the extracellular levels of dopamine compared with values with vehicle (Fig. 2).

Local application of GBR 12935 in the nucleus accumbens increased the extracellular levels of dopamine to approximately 400% of basal. Co-application of 100 μm sulpiride or raclopride with 100 μm GBR 12935 did not

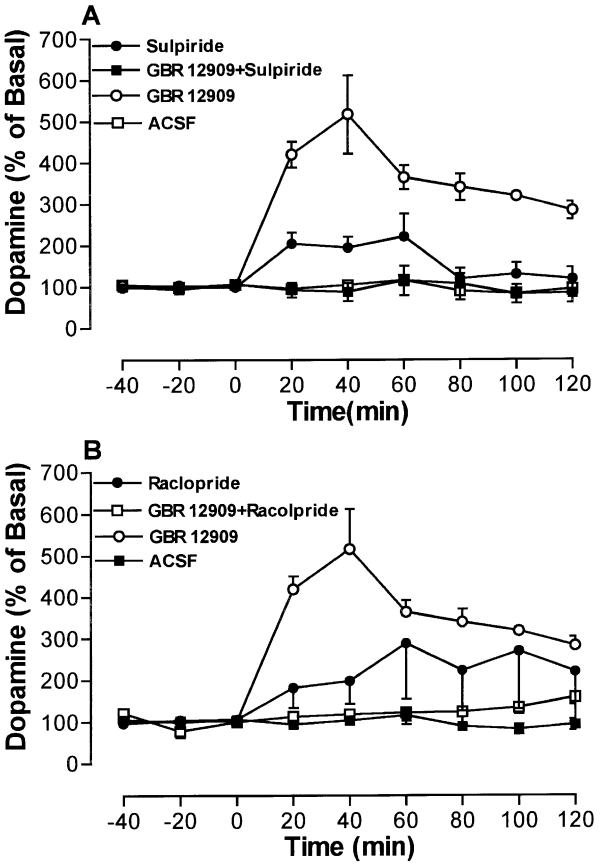

Fig. 2. (A) Effects of local perfusion for 60 min with artificial cerebrospinal (ACSF) fluid alone, 100 μM sulpiride alone, 100 μM GBR 12909 alone, and 100 μM GBR 12909 plus 100 μM sulpiride on the extracellular levels of dopamine in the nucleus accumbens. All agents were perfused starting at the zero time point. Data are mean ± SEM values of five animals for each treatment. A two-way ANOVA (treatment × time) with repeated measures revealed a significant effect of treatment $F(3,16) = 45.75$, $P < 0.001$, and time, $F(6,11) = 14.72$, $P < 0.001$. There was a significant interaction $F(18,32) = 4.12$, $P < 0.01$. The planned comparisons (LSD) revealed that there was a significant difference ($P < 0.05$) between treatment of sulpiride or GBR 12909 alone vs. ACSF, and GBR 12909 alone vs. GBR 12909 plus sulpiride. The basal extracellular levels of dopamine in these groups were 34 ± 5 fmol/20 min. (B) Effects of local perfusion for 60 min with ACSF alone, 100 μM raclopride alone, 100 μM GBR 12909 alone, and 100 μM GBR 12909 plus 100 μM raclopride on the extracellular levels of dopamine in the nucleus accumbens. Data are mean ± SEM values of four to five animals for each treatment. A two-way ANOVA (treatment × time) with repeated measures revealed a significant effect of treatment $F(3,15) = 7.89$, $P < 0.001$, and time, $F(6,10) = 9.88$, $P < 0.001$. There was a significant interaction $F(18,29) = 2.39$, $P < 0.02$. The planned comparisons (LSD) revealed that there was a significant difference ($P < 0.05$) between treatment of GBR 12909 alone vs. ACSF, and GBR 12909 alone vs. GBR 12909 plus raclopride. The basal extracellular levels of dopamine in these groups were 26 ± 2 fmol/20 min.

alter the extracellular levels of dopamine compared with levels for artificial cerebrospinal fluid (Fig. 3).

To examine a possible negative interaction of GBR uptake inhibitors with antagonists for other dopamine receptors, a study was conducted to determine the effects of co-perfusion of a dopamine D1 receptor antagonist SCH 23390 with GBR 12909 on extracellular levels of dopamine (data not shown). Local perfusion of nucleus accumbens with 100 μm SCH 23390 increased the extracellular levels of dopamine in the nucleus accumbens to approximately 180% of basal. In contrast to the dopamine D2 receptor antagonists, co-application of 100 μm SCH 23390 with GBR 12909 increased the extracellular levels of dopamine to 404 ± 46% ($n = 5$) of basal.

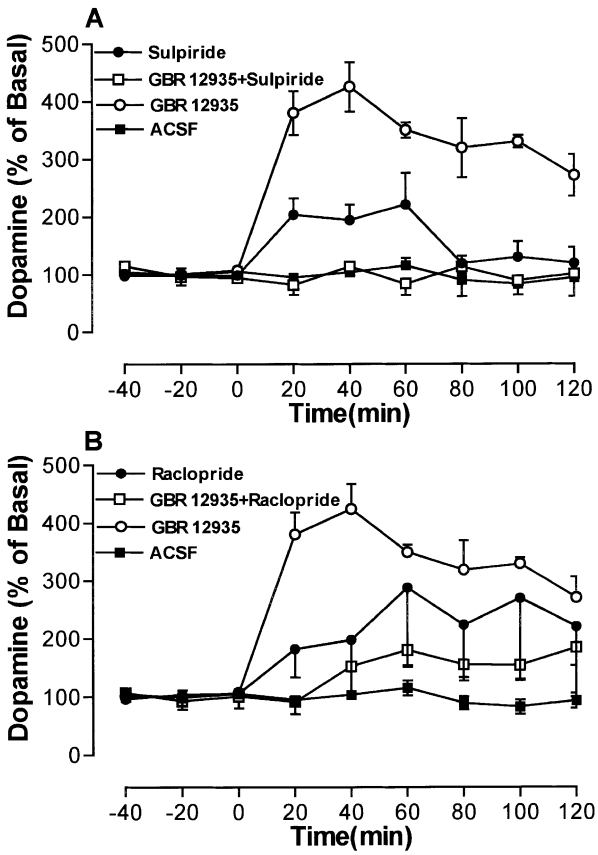

Fig. 3. (A) Effects of local perfusion for 60 min with ACSF, 100 μM sulpiride alone, 100 μM GBR 12935 alone, and 100 μM GBR 12935 plus 100 μM sulpiride on the extracellular levels of dopamine in the nucleus accumbens. Data are mean ± SEM values of three to five animals for each treatment. All agents were perfused starting at the zero time point. A two-way ANOVA (treatment × time) with repeated measures revealed a significant effect of treatment $F(3,13) = 41.83$, $P < 0.001$, and time, $F(6,8) = 22.80$, $P < 0.001$. There was a significant interaction $F(18,23) = 3.22$, $P < 0.05$. The planned comparisons (LSD) revealed that there was a significant difference ($P < 0.05$) between treatment of sulpiride or GBR 12935 alone vs. ACSF, and GBR 12935 alone vs. GBR 12935 plus sulpiride. The basal extracellular levels of dopamine in these groups were 32 ± 3 fmol/20 min. (B) Effects of local perfusion for 60 min with ACSF alone, 100 μM raclopride alone, 100 μM GBR12935 alone, and 100 μM GBR 12935 plus 100 μM raclopride on the extracellular levels of dopamine in the nucleus accumbens. Data are mean ± SEM values of three to five animals for each treatment. A two-way ANOVA (treatment × time) with repeated measures revealed a significant effect of treatment $F(3,12) = 3.73$, $P < 0.05$, and time, $F(6,7) = 17.11$, $P < 0.01$. There was a significant interaction $F(18,20) = 3.06$, $P < 0.01$. The planned comparisons (LSD) revealed that there was a significant difference ($P < 0.05$) between treatment of GBR 12935 alone vs. ACSF, and GBR 12935 alone vs. GBR 12935 plus raclopride The basal extracellular levels of dopamine in these groups were 28 ± 3 fmol/20 min.

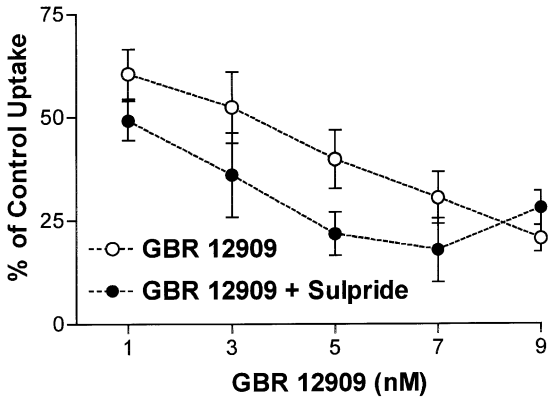

Fig. 4. Effect of 100 μM sulpiride on the inhibition of [^{3}H]dopamine uptake by 1–9 nM GBR 12909 in vitro. Nucleus accumbens homogenates were incubated at 30°C for 2 min in the presence of 10 nM [^{3}H]dopamine with GBR 12909 alone or GBR 12909 plus sulpiride. One-way ANOVA revealed a significant treatment effect of GBR 12909 with sulpiride $F(4,60) = 22.3$, $P < 0.05$. Sulpiride significantly increased the effect of GBR 12909 $F(4,60) = 3.18$, $P < 0.05$. Values are presented as mean percent of control uptake. The mean ± SEM control value of specific uptake was 5.2 ± 0.5 pmol/mg protein/2 min for $n = 6$ determinations.

To examine the possibility that co-perfusion of the GBR-type dopamine uptake inhibitors with dopamine D2 receptor antagonists might interact with the dialysis membrane itself and reduce diffusion through the membrane, an in vitro dialysis experiment was conducted to examine recovery of dopamine with 100 μm GBR 12909 alone and 100 μm GBR 12909 plus 100 μm sulpiride. There was no significant differences in the recovery of dopamine with artificial cerebrospinal fluid (10.4%), GBR 12909 (10.3%) or GBR 12909 plus sulpiride (9.6%). Therefore, it does not appear that recovery of dopamine was affected with the combination.

3.3. [^{3}H]dopamine uptake

An interaction of a dopamine D2 receptor antagonist with a GBR compound on the inhibition of dopamine uptake was studied in synaptosomal homogenates prepared from the nucleus accumbens. The concentrations of GBR 12909 used in the in vitro assay were based upon previous studies with dopamine uptake inhibitors (Simon et al., 1997). GBR 12909 (1–9 nM; Fig. 4) dose-dependently inhibited [^{3}H]dopamine uptake in the nucleus accumbens homogenates. Instead of blocking the effects of GBR, addition of 100 μm sulpiride produced a small but significant increase in inhibition of [^{3}H]dopamine uptake (Fig. 4). Sulpiride alone had no significant effect on dopamine uptake (100 ± 5%).

4. Discussion

The major finding of this study suggests that an interaction occurs in vivo between selective dopamine D2 receptor antagonists (sulpiride and raclopride) and GBR-type dopamine uptake inhibitors, which results in the inhibitory effects on dopamine reuptake and on the dopamine D2 autoreceptor being negated when both compounds are co-perfused (Figs. 2 and 3). These results are contrary to the results obtained with concurrent perfusion with nomifensine and sulpiride, which produced a greater elevation in the extracellular levels of dopamine in the nucleus accumbens than was produced by either compound alone (Rahman and McBride, 2000).

Local application of sulpiride or raclopride alone increased the extracellular levels of dopamine in the nucleus accumbens (Figs. 2 and 3). Activation of dopamine D2 receptors suppresses the functional activity of dopamine neurons at various critical sites, i.e. impulse generation, dopamine synthesis, and dopamine release (Wolf and Roth, 1987), whereas inhibiting dopamine D2 autoreceptors has the opposite effect. The elevation of extracellular levels of dopamine with local application of dopamine D2 receptor antagonists suggests that blocking dopamine D2 nerve terminal autoreceptors is the likely mechanism underlying this effect. This finding is in agreement with previous studies with sulpiride (Mendlin et al., 1998; Rahman and McBride, 2000). These observations with dopamine D2 receptor antagonists are also in conformity with the results of previous microdialysis studies in a number of dopamine target regions (Imperato and Di Chiara, 1988; Westerink and De Vries, 1989; Santiago et al., 1993).

Local perfusion of the dopamine uptake inhibitors GBR 12909 or GBR 12935 alone through the dialysis probe in the nucleus accumbens increased synaptic dopamine levels. These data are consistent with the findings using in vivo microdialysis in the nucleus accumbens (Engleman et al., 2000; Kohl et al., 1998; Li et al., 1996; Rahman and McBride, 2000) or other dopamine target regions (Carboni et al., 1989; Nomikos et al., 1990; Nakaachi et al., 1995; Engberg et al., 1997). Rahman and McBride (2000) previously reported that a dose–response effect for GBR 12909 on the extracellular levels of dopamine in the nucleus accumbens was obtained over the range of 10–1000 μm. The 100 μm concentration was chosen in the present study because dopamine concentrations were elevated to a level which allowed both increases and decreases to be easily measured (Rahman and McBride, 2000). The concentrations used in the present study are similar to concentrations of dopamine uptake inhibitors used in other studies (Engleman et al., 2000; Kalivas and Duffy, 1991; Kohl et al., 1998; Li et al., 1996; Nomikos et al., 1990). High concentrations need to be perfused through the microdialysis probe because of the low efficiency of diffusion across the dialysis membrane and away from the probe. Therefore, the concentration reaching the tissue is significantly lower than the concentration in the probe. However, the tissue concentration of dopamine uptake inhibitors is unknown, and non-specific effects of the agents perfused cannot be ruled out.

Co-perfusion with raclopride or sulpiride significantly reduced the effects of GBR 12909 or GBR 12935 on elevating the extracellular levels of dopamine in the nucleus accumbens (Figs. 2 and 3). The mechanisms underlying this negative interaction are unknown but may be a result of an action of the dopamine D2 receptor antagonists at the GBR binding site on the dopamine transporter (Cass and Gerhardt, 1994) and an action of the GBR compounds at the binding site for the antagonists on the dopamine D2 autoreceptor (Andersen, 1989). An inhibition of GBR binding by sulpiride at the dopamine transporter does not appear to account for the negative interaction of sulpiride on the GBR-induced elevation in the extracellular levels of dopamine because sulpiride had little effect on GBR 12909 inhibition of [^{3}H]dopamine uptake (Fig. 4). In addition, inhibition of sulpiride binding by GBR 12909 at the dopamine D2 autoreceptor would not account for the complete negation of the effects of both compounds. Alternatively, the dopamine D2 antagonist and GBR compounds could be acting together at unknown sites (e.g., ion channels) to inhibit release of dopamine. Inhibiting the release of dopamine would prevent observing any marked effects of blocking dopamine uptake or inhibiting the dopamine D2 autoreceptor. The negative interaction between the dopamine D2 receptor antagonists and dopamine uptake blockers may be unique to GBR-type compounds because a positive interaction has been reported for co-perfusion of sulpiride and nomifensine (Rahman and McBride, 2000).

There are number of possible ways in which the effects of GBR compounds could be modulated by dopamine D2 receptor antagonists. It has been shown that GBR 12909 has low potency or no effect on inducing dopamine release (Van der Zee et al., 1980; Bonnet and Costentin, 1986; Andersen, 1987; Herdon et al., 1987). Furthermore, GBR 12909 is known to have a 100-fold greater potency (IC_{50} 1 nM) than that of other dopamine uptake blockers, e.g., nomifensine (IC_{50} 134 nM), for inhibiting dopamine uptake (Andersen, 1989). Besides inhibiting dopamine uptake, GBR 12909 also has affinity for dopamine D2 receptors (IC_{50} 270 nM) as compared to nomifensine ($>$ 10,000 nM). Moreover, GBR 12909 has a high affinity for voltage dependent sodium channels, as labelled by [^{3}H]batrachotoxinin (Andersen, 1989). Furthermore, GBR compounds possess high affinity for a piperazine binding site in the brain and this site may be allosterically coupled with [^{3}H]batrachotoxinin binding site, as suggested by Andersen (1989). Phosphorylation of these sites or other possible sites present on the dopamine transporter may alter dopamine transporter function that lead to changes in dopamine uptake inhibition, as dopamine D2 receptors are known to regulate the dopamine synthesis via phosphorylation (Salah et al., 1989). In light of this evidence, it may be concluded that GBR compounds have a unique neurochemical profile in addition to dopamine uptake inhibition. Whether these neurochemical actions may account for the negative interactions with selective dopamine D2 receptor antagonists in preventing the elevation in the extracellular levels of dopamine in the nucleus accumbens remains to be clarified. However, the higher affinity of GBR 12909 than nomifensine for inhibiting dopamine uptake is not a likely explanation for sulpiride enhancing the effects of nomifensine on the extracellular levels of dopamine in the nucleus accumbens (Rahman and McBride, 2000) but inhibiting the actions of GBR 12909 in the present study (Fig. 2). If sulpiride was acting at the dopamine transporter to interfere with the action of the uptake inhibitors, then sulpiride might be expected to produce a larger effect on nomifensine than GBR 12909 on the basis of their relative potencies at the dopamine transporter.

The effect of sulpiride on GBR compounds is not likely due to a decrease of dopamine release (Herdon et al., 1987). Because 100 μm sulpiride in vitro enhanced rather than reduced the effect of GBR 12909 to inhibit dopamine uptake, the effect of co-perfusion with dopamine D2 antagonists in vivo may not occur through a direct effect on the ability of GBR to inhibit dopamine uptake. The apparent disparity between the results from the in vivo microdialysis and the in vitro uptake studies cannot be easily explained. In vivo changes in the extracellular concentration of dopamine are regulated by (a) phasic release from axon terminals due to depolarization (Grace, 1991), (b) tonic control from axon terminals (Grace, 1991), (c) uptake into neuronal and non-neuronal sites (Wieczorek and Kruk, 1994), and (d) activation of dopamine D2 autoreceptors which can modulate phasic release. In addition, excitatory and inhibitory inputs at the level of the cell body and axon terminal also regulate release. It is possible that GBR-type dopamine uptake inhibitors and dopamine D2 receptor antagonists are also acting together to reduce excitatory and/or increase inhibitory regulation of the dopamine system, thereby reducing dopamine release and attenuating any effects on blocking dopamine uptake or D2 autoreceptor inhibition.

In conclusion, the results of the present study indicate that complex negative interactions between GBR-type dopamine uptake inhibitors and dopamine D2 receptor antagonists occur in vivo and dopamine D2 receptor antagonists are capable of modulating the extracellular dopamine levels. However, the underlying mechanisms by which dopamine D2 receptor antagonists can alter the activity of GBR-type compound remain to be established.

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