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Effect of tolcapone, a catechol-*O*-methyltransferase inhibitor, on striatal dopaminergic transmission during blockade of dopamine uptake

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Abstract

To examine the mechanisms of tolcapone in the central nervous system (CNS), we analyzed alterations in parameters of striatal dopamine transmission induced by this drug (30 mg/kg) co-administered with the selective dopamine uptake inhibitor, GBR 12909 (10 mg/kg). Using microdialysis in freely moving rats, it was determined that combined administration of tolcapone with GBR 12909 resulted in a further increase of dopamine levels over that obtained without the catechol-*O*-methyltransferase inhibitor, while tolcapone alone failed to change dopamine levels. Fast-scan cyclic voltammetric monitoring of electrically evoked dopamine did not show any changes in dopamine release after the combination of the drugs, but there was a pronounced decrease in the rate of dopamine clearance after GBR 12909 alone and when co-administered with tolcapone. These data indicate that in rat striatum, a tolcapone-induced increase in extracellular dopamine is not observed because of the presence of uptake. These results also support the hypothesis that under normal conditions, uptake, rather than metabolism, control extracellular levels of dopamine.

Keywords: Dopamine release; Dopamine uptake; Dopamine synthesis; Microdialysis; Voltammetry, cyclic; Tolcapone; GBR 12909

1. Introduction

One of the modern pharmacological approaches for the treatment of Parkinson's disease is to optimize the effect of 3,4-dihydroxyphenyl-L-alanine (L-DOPA) by inhibiting dopamine catabolism via blockade of catechol-*O*-methyltransferase (Männistö and Kaakkola, 1990; Kaakkola et al., 1994; Gottwald et al., 1997). As a part of this strategy, several selective and potent catechol-*O*-methyltransferase inhibitors have been recently developed (Da Prada et al., 1991; Kaakkola et al., 1994; Gottwald et al., 1997). For example, tolcapone (Ro 40-7595) has been used as an adjunct therapy with L-DOPA in patients with Parkinson's disease (Limousin et al., 1995; Gottwald et al., 1997; Waters et al., 1997). Like other catechol-*O*-methyltransferase inhibitors, tolcapone reduces the peripheral formation of 3-*O*-methyl-DOPA from L-DOPA, thereby increasing the bioavailability of L-DOPA (Zürcher et al., 1990a,b, 1993; Sedek et al., 1997). Moreover, because tolcapone can cross the blood–brain barrier, it decreases the metabolism of striatal extracellular dopamine by inhibition of its conversion to 3-methoxytyramine (Männistö et al., 1992). As a result of these dual actions, tolcapone markedly potentiates the effect of L-DOPA on dopamine extracellular levels. This potentiation leads to a considerable benefit in the treatment of Parkinson's disease.

Surprisingly, in microdialysis studies, tolcapone itself does not alter extracellular dopamine levels, but it does exert potent effects on dopamine metabolite levels (Napolitano et al., 1995; Budygin et al., 1997a,b, 1998). It is known that extracellular dopamine dynamics is potently regulated by the active inward transport of neurotransmitter by the dopamine transporter. The relative contribution of the dopamine transporter and catechol-*O*-methyl-

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transferase in dopamine clearance is not well-studied. However, findings in dopamine transporter knockout mice suggest that catechol-*O*-methyltransferase plays a relatively insignificant role in comparison to uptake (Jones et al., 1998).

To further examine the mechanisms of the central action of tolcapone, we analyzed alterations in striatal dopamine transmission induced by combined blockade of both catechol-*O*-methyltransferase and the dopamine transporter. Using in vivo neurochemical approaches, we studied the effects of tolcapone co-administered with the selective dopamine uptake inhibitor, GBR 12909, on dopamine synthesis, release and metabolism. The hypothesis tested is that uptake, rather than metabolism, normally controls extracellular levels of dopamine. The results support this hypothesis since extracellular dopamine levels are elevated by tolcapone only when uptake is inhibited.

2. Materials and methods

2.1. Animals and drugs

Male Sprague–Dawley rats (250–300 g) were purchased from Charles River (Raleigh, NC) and housed under controlled temperature and lighting conditions. Food and water were available ad libitum. Animal care was in accordance with the Guide for Care and Use of Laboratory Animals (NIH Publication 86-23) and was approved by the Institutional Animal Care and Use Committee of the University of North Carolina.

Tolcapone (Ro 40-7595; Hoffmann-La Roche, Basel, Switzerland) suspended in saline containing 1% Tween 80 using a glass homogenizer was administered at a dosage of 30 mg/kg. GBR 12909 hydrochloride and NSD 1015 (RBI, Natick, MA) were dissolved in 0.9% saline before injection at a dosage of 10 and 50 mg/kg, respectively. All drugs were administered intraperitoneally in a final volume of 2 ml/kg.

2.2. HPLC assessment of brain content of monoamines and metabolites

The striata of adult rats were homogenized in 0.1 M $HClO_4$ containing 100 ng/ml 3,4-dihydroxybenzylamine as an internal standard. Homogenates were centrifuged for 10 min at $10{,}000 \times g$. Supernatants were filtered through 0.22 μm filters and analyzed for levels of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) using high performance liquid chromatography with electrochemical detection (HPLC-EC). Dopamine and metabolites were separated on a reverse-phase column (C-18, 5 μm, 100×4.60 mm, Ultremex) with a mobile phase consisting of 0.03 M citrate-phosphate buffer with 2.1 mM octyl sodium sulfate, 0.1 mM EDTA, 10 mM NaCl and 17% methanol (pH 3.6) at a flow rate of 1 ml/min and detected by a glass carbon electrode (BAS) set at +0.8 V. The volume of injection was 20 μl. Determinations of L-DOPA in striatal homogenates were performed using the same column and apparatus with a mobile phase consisting of 50 mM monobasic sodium phosphate, 0.2 mM octyl sodium sulfate, 0.1 mM EDTA, 10 mM NaCl and 10% methanol (pH 2.6). The potential applied was +0.65 V.

2.3. Determination of monoamine synthesis rates in vivo

To measure dopamine synthesis rates, rats were injected with the L-aromatic acid decarboxylase inhibitor, 3-hydroxybenzylhydrazine (NSD-1015), 50 mg/kg, i.p. Tolcapone, GBR 12909, and saline were administered 10 min before NSD 1015 (Carlsson et al., 1972). Fifty minutes later, the concentration of striatal L-DOPA was determined using HPLC-EC for the measurement of tyrosine hydroxylase activity. The measurements were performed using the chromatographic conditions and mobile phase as described above.

2.4. In vivo microdialysis

Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic frame. Dialysis probes (4 mm membrane length, 0.24 mm, Cuprophane, 6 kDa cut-off, CMA-10, CMA/Microdialysis, Solna, Sweden) with CMA-10 guide cannulae were implanted into the right striatum. The stereotaxic coordinates for implantation of microdialysis probes were (in mm): +0.4 AP, −3.0 ML, −6.5 DV (Paxinos and Watson, 1986) relative to bregma. Placement of the probe was verified by histological examination subsequent to the experiments.

Following surgery, animals were returned to their home cages with free access to food and water. Twenty four hours after surgery, the dialysis probe was connected to a syringe pump and perfused at 1 μl/min with artificial cerebrospinal fluid (composition in mM: Na^+ 150, K^+ 3.0, Ca^{2+} 1.0, Mg^{3+} 1.2, PO_4^{3-} 1.0, Cl^- 129, HCO_3^- 25, pH 7.3). After a 1-h equilibration period, the perfusates were collected every 20 min. At least three control samples were taken before drugs were administered. Perfusate samples were assayed for dopamine using HPLC-EC under the chromatographic conditions described above.

2.5. Behavioral activity assessment

Immediately before and at intervals after drug administration, animals were assessed for stereotyped behavior. For this procedure, each rat was observed individually for a 5-s period at 1-min intervals over 5 consecutive min. The data are expressed as stereotypy scores, using a conventional 0–6 point stereotypy scale (Murray and Waddington, 1990; Gainetdinov et al., 1997). The testing was repeated at 20-min intervals.

2.6. Electrochemistry

Carbon-fiber electrodes were prepared as described previously (Kawagoe et al., 1993). The electrode potential was linearly scanned from −0.4 V to 1.0 V and back to −0.4 V at 300 V/s every 100 ms. To convert the magnitude of current at the oxidation potential for dopamine (usually between 0.5 and 0.7 V) to concentration, each electrode was calibrated with a known concentration of dopamine at the end of the experiment. Current measured at the oxidation potential for dopamine is directly proportional to the dopamine concentration.

All voltammetry experiments were performed in anesthetized animals. The surgical procedure, stimulation parameters and apparatus have been described in detail in previous publications (Wightman and Zimmerman, 1990; Kawagoe et al., 1993; Garris et al., 1997). Twisted, bipolar stimulating electrodes were positioned in the medial forebrain bundle (−4.6 AP, −1.4 ML, −8.5 DV) and Nafion-coated or cylinder carbon-fiber microelectrodes (Baur et al., 1988) were positioned in the striata (+1.2 AP, −2.0 ML, −3.5 DV), according to their stereotaxic coordinates as represented in the Atlas of Paxinos and Watson (1986). The reference electrode was a sodium-saturated calomel electrode that was in contact with dura via a salt bridge.

Cyclic voltammograms were recorded at the carbon-fiber electrode every 100 ms at a scan rate of 300 V/s using a bipotentiostat (EI400, Ensman Instruments, Bloomington, IN). Data were digitized (DMA Labmaster, Scientific Solutions, Solon, OH) and stored to computer files.

2.7. Voltammetry data analysis

Dopamine uptake was determined from the clearance rate of dopamine following the termination of the stimulus. Uptake was assumed to follow the Michaelis–Menten equation, and the change in dopamine during and after stimulated release was modeled as:

$$d[DA]/dt = (f)[DA]_p - \left(V_{max}/\{(K_m/[DA]) + 1\}\right),$$

where f is the stimulation frequency (Hz), $[DA]_p$ is the concentration of dopamine released per stimulus pulse, and V_{max} is the maximal rate of dopamine uptake. V_{max} was measured from the descending slope of the curve following a 60-Hz, 2-s stimulation, a condition in which $[DA] \gg K_m$. The value of K_m was taken to be 0.16 μM, a value determined in rat brain synaptosomes (Near et al., 1988).

The derivative form of the above equation was used to simulate the dopamine response (Kawagoe et al., 1992). The response time of the electrode, determined during post-calibration, was used to convolute the effects of the Nafion film thickness with the simulation (Kawagoe et al., 1992). After the V_{max} and K_m values were determined, they were kept constant while the value of $[DA]_p$ was adjusted until the best fit for the simulation was obtained] based on the correlation coefficient (r) between simulation and data]. When uptake inhibitors were administered, the pre-drug V_{max} and $[DA]_p$ values were kept constant while the K_m value was adjusted to the best fit. Once the post-drug K_m value had been determined, the $[DA]_p$ was adjusted until the maximal correlation coefficient was obtained.

2.8. Data analysis

The data are presented as mean ± S.E.M. and were analyzed statistically by ANOVA with Fisher's protected least-significant difference test. Differences with $P < 0.05$ are reported.

3. Results

3.1. Effects of GBR 12909 and tolcapone administered alone and in combination on striatal dopamine, DOPAC and HVA tissue content

As shown in Fig. 1, the administration of GBR 12909 (10 mg/kg, i.p.) did not alter dopamine and HVA tissue content, but decreased slightly (20%) DOPAC concentration. Tolcapone (30 mg/kg, i.p.) also did not alter dopamine tissue content, but in contrast to GBR 12909, it increased DOPAC concentrations (80%) and decreased HVA concentration (60%) ($P < 0.05$). The co-administration of tolcapone and GBR 12909 resulted in an increase in the tissue content of DOPAC concentration (30%) although the increase was to a lesser degree when compared to tolcapone alone ($P < 0.05$). However, the combination of drugs changed dopamine and HVA concentrations similarly to that found for tolcapone alone. Control values for dopamine, DOPAC, and HVA were—9.12 ± 0.35, 0.76 ± 0.89 and 0.16 ± 0.02 ng/mg tissue, respectively.

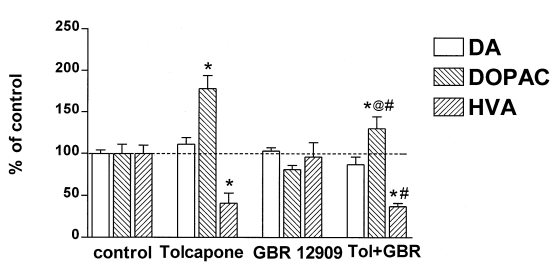

Fig. 1. Effect of tolcapone (30 mg/kg, i.p., 1 h before the rats were killed) and GBR 12909 (10 mg/kg i.p., 1 h before the rats were killed), alone or in combination, on the dopamine, DOPAC and HVA concentrations. Shown are means ± S.E.M. of four rats, expressed as percent of saline-treated controls. $^{*}P < 0.05$ vs. controls, $^{\#}P < 0.05$ vs. GBR, $^{@}P < 0.05$ vs. tolcapone.

3.2. Effects of GBR 12909 and tolcapone administered alone and in combination on striatal L-DOPA accumulation

As shown in Fig. 2, the administration of GBR 12909 (10 mg/kg, i.p.) decreased the NSD 1015-induced accumulation of tissue levels of L-DOPA to 73 ± 10% of that found with NSD + saline-treated controls (2.61 ± 0.11 ng/mg tissue) ($P < 0.05$). Similar results were obtained by Nissbrandt et al. (1991). In contrast, tolcapone (30 mg/kg, i.p.) produced an increase in L-DOPA levels to 131 ± 11% ($P < 0.05$). However, when tolcapone was co-administered with GBR 12909, L-DOPA levels decreased to 54 ± 8% of control ($P < 0.05$).

3.3. Effects of GBR 12909 and tolcapone administered alone and in combination on striatal extracellular levels of dopamine, DOPAC and HVA

Using microdialysis in freely moving rats, it was determined that striatal extracellular levels of dopamine were unchanged after tolcapone (30 mg/kg, i.p.), but were increased ($P < 0.05$) after GBR 12909 (10 mg/kg, i.p.). The combined administration of tolcapone (30 mg/kg, i.p.) and GBR 12909 (10 mg/kg, i.p.) resulted in a further increase ($P < 0.05$) of dopamine levels over that obtained without the COMT inhibitor (Fig. 3). It was reported earlier that tolcapone (30 mg/kg, i.p.) significantly decreased HVA and increased DOPAC levels in striatal dialysate (Napolitano et al., 1995; Budygin et al., 1997a,b, 1998), whereas GBR 12909 (10 mg/kg, i.p.) produced no significant effect on these metabolites (Westerink et al., 1987; Budygin et al., 1997a,b). The co-administration of tolcapone and GBR 12909 did not result in any changes in extracellular DOPAC and HVA concentrations, compared to the effect of tolcapone alone (data not shown).

3.4. Effects of tolcapone on GBR 12909-induced activation of stereotypy

Tolcapone was without any significant effect on stereotypy (Fig. 4). However, tolcapone markedly potentiated GBR 12909-induced activation. Maj et al. (1990) have reported similar effects of tolcapone on amphetamine- and nomifensine-induced hyperactivity and stereotypy.

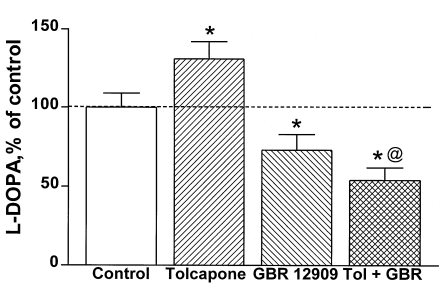

Fig. 2. Effect of tolcapone (30 mg/kg, i.p., 10 min before NSD 1015) and GBR 12909 (10 mg/kg, i.p., 10 min before NSD 1015), alone or in combination, on the 3,4-dihydroxyphenylalanine (DOPA) accumulation following NSD 1015 administration (50 mg/kg, i.p., before the rats were killed). Shown are means ± S.E.M. of four rats, expressed as percent of NSD 1015-treated controls. $^{*}P < 0.05$ vs. controls, $^{@}P < 0.05$ vs. tolcapone.

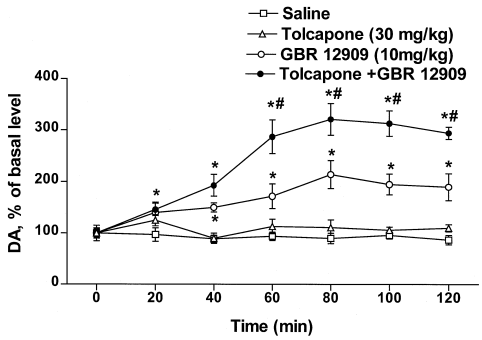

Fig. 3. Effect of tolcapone (30 mg/kg) and GBR 12909 (10 mg/kg), alone or in combination, on striatal dialysate levels of dopamine. Data are means ± S.E.M. of five to six rats per group, expressed as percent of basal output (average of the three samples before drug administration). $^{*}P < 0.05$ vs. saline-treated controls, $^{\#}P < 0.05$ difference from GBR 12909-treated group. All of the points representing the effect of the drugs administered in combination (except for the effect measured 20 min after administration) were significantly different ($P < 0.05$) from the tolcapone group.

3.5. Effects of GBR 12909 and tolcapone administered alone and in combination on the parameters of dopamine release and uptake in rat striatum (in vivo voltammetry)

No significant difference was observed in the value of $[DA]_p$ (Fig. 5A) and V_{max} (data not shown) measured prior to and after administration of GBR 12909 (10 mg/kg, i.p.) and tolcapone (30 mg/kg, i.p.) administered alone and in combination. However, there was a 20-fold increase in K_m after GBR 12909 (from 160 nM to 3171 ± 709 nM) that is associated with a decrease in the rate of disappearance of dopamine from the synaptic cleft (Fig. 5B). This result supports the belief that GBR 12909 is a specific dopamine uptake inhibitor without a transmitter-releasing action. This conclusion differs from one we made previously (Maj et

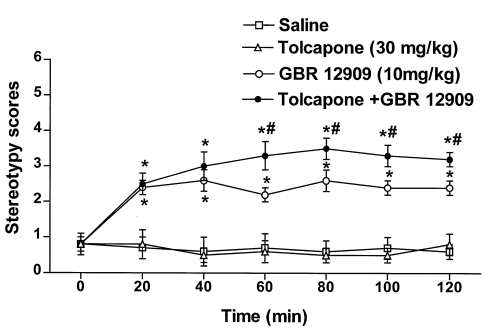

Fig. 4. Effect of tolcapone (30 mg/kg) and GBR 12909 (10 mg/kg), alone or in combination, on stereotyped behavior rats. Data are means ± S.E.M. of six rats. $^{*}P < 0.05$ vs. saline-treated controls, $^{\#}P < 0.05$ difference from GBR 12909-treated group. All of the points representing the effect of drugs administered in combination were significantly different ($P < 0.05$) from the tolcapone group.

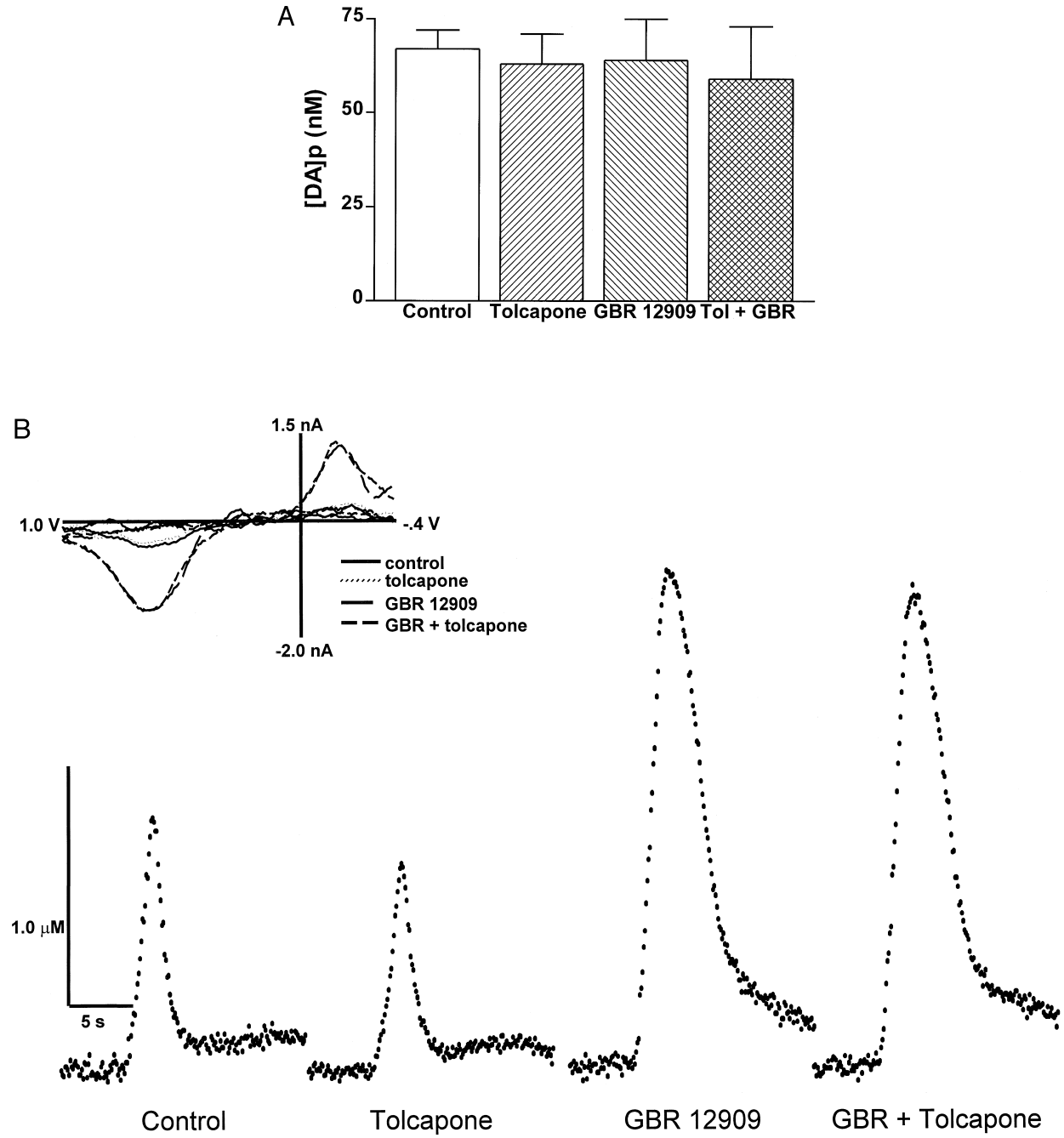

Fig. 5. Effect of tolcapone (30 mg/kg) and GBR 12909 (10 mg/kg), alone or in combination, on dopamine overflow measured by in vivo voltammetry (60 Hz, 2s stimulation). (A) $[DA]_p$ (the concentration of dopamine released per stimulus pulse). Data are means ± S.E.M. of four rats per group. (B) Temporal responses in a single animal.

al., 1988); however, improved analysis methods were used here, leading to greater credence in these conclusions.

4. Discussion

The present study performed with tolcapone (30 mg/kg, i.p.) and GBR 12909 (10 mg/kg, i.p.) demonstrate potentiation of dopaminergic neurotransmission by a combination of uptake blockade and enzyme inhibition (Fig. 3). These observations are in line with previous reports, demonstrating that tolcapone does not alter dopamine extracellular levels (Napolitano et al., 1995; Budygin et al., 1997a,b, 1998), but potentiates the nomifensine (15 mg/kg) -induced increase of striatal dopamine in anaesthetized rats (Kaakkola and Wurtman, 1992). Furthermore, in a study with freely moving rats, similar results were obtained when tolcapone (30 mg/kg, i.p.) was administered in combination with GBR 12909 (5 μM intrastriatal or 20 mg/kg, i.p.) (Budygin et al., 1997a,b)

There are several processes by which tolcapone can increase dopamine extracellular levels. For example, tolcapone can elevate extracellular dopamine by inhibiting dopamine metabolism to 3-methoxytyramine (Männistö et al., 1992). In addition, tolcapone's inhibition of peripheral and central L-DOPA-*O*-methylation results in the elevation of dopamine precursor levels in the brain (Zürcher et al., 1990a,b, 1993), which may lead to an increase in dopamine biosynthesis and possibly an enhancement of dopamine release. In fact, our data demonstrate a modest, but significant, tolcapone-induced increase in L-DOPA accumulation following NSD 1015, an index of increased dopamine synthesis (Fig. 2). Despite this increase in synthesis and decrease in dopamine metabolism, we were unable to observe any tolcapone-induced changes in extracellular dopamine levels using microdialysis (Fig. 3). This result might be interpreted to mean that an increase in dopamine synthesis after tolcapone does not translate to an elevation in dopamine release or that uptake mechanisms prevent any increase in dopamine extracellular levels caused by tolcapone. In the last case, increased dopamine overflow as a consequence of increased dopamine synthesis might be undetectable by microdialysis due to relatively low temporal resolution (20-min sampling).

However, using fast-scan cyclic voltammetry with a 100-ms temporal resolution, neither tolcapone alone nor when co-administered with GBR 12909 had an effect on $[DA]_p$, an indicator of synaptic dopamine release (Fig. 5). Thus, it appears that tolcapone's ability to moderately increase dopamine synthesis, presumably through an increase in endogenous L-DOPA availability, does not result in an appreciable modulation of dopamine release. Rather in the absence of exogenous L-DOPA, the drug appears to have mostly central effects that modify dopamine metabolism causing changes in striatal DOPAC and HVA content. Since extracellular levels are more strongly governed by uptake than metabolism, no detectable change in dopamine overflow is seen. However, when uptake rates are slowed pharmacologically, the effects of tolcapone on extracellular dopamine are observed. Dopamine levels are elevated by tolcapone since extracellular metabolism becomes a more important control mechanism under these conditions. Furthermore, these results suggest that the increase in dopamine overflow seen following the co-administration of L-DOPA and tolcapone is primarily a result of the inhibition of peripheral catechol-*O*-methyltransferase.

These neurochemical conclusions are consistent with our behavioral observations (Fig. 4). Tolcapone was without any significant effect on the measured behavioral parameters. This result is not consistent with studies by Maj et al. (1990), who reported that tolcapone (10 and 20 mg/kg) increased the exploratory activity of rats. However, the stereotypy scores used in this study might be a less sensitive measure of detecting subtle changes in behavior. GBR 12909 produced moderate activation that was markedly potentiated when GBR 12909 and tolcapone were administered together. It should be noted that Maj et al. (1990) has reported similar effects of tolcapone on amphetamine- and nomifensine-induced hyperactivity and stereotypy. These data demonstrate that dopamine transmission can be potentiated by the inhibition of catechol-*O*-methyl-transferase only when dopamine clearance is also blocked.

According to previous reports (Nissbrandt et al., 1991), GBR 12909 has a slightly inhibitory effect on dopamine biosynthesis rates (Fig. 2) that is probably secondary to an increased amount of dopamine in the synaptic cleft, resulting in activation of synthesis-modulating autoreceptors. Since tolcapone potentiates the GBR 12909-induced increase in dopamine overflow, it seems reasonable that a combination of the drugs results in a greater activation of synthesis-inhibiting autoreceptors and a more pronounced decrease in L-DOPA levels. It is important to note once more that tolcapone alone produces a significant increase in dopamine synthesis rates, which presumably reflects an increased brain L-DOPA availability due to tolcapone's inhibitory effect on peripheral formation of 3-*O*-methyl-DOPA. This observation parallels the fact that a combination of GBR 12909 and tolcapone did not have as great of an affect on DOPAC levels in comparison to tolcapone alone (Fig. 1), suggesting that in healthy brain, autoreceptor-mediated control of dopamine synthesis is more effective than control via precursor availability. If this hypothesis is true, then the potentially beneficial effect of the combination of tolcapone and GBR 12909 on increasing dopamine extracellular levels might be short-acting and not useful in chronic drug administration.

In summary, dopamine re-uptake through the dopamine transporter seems to be the major competitor that keeps tolcapone from increasing central dopamine transmission.

Acknowledgements

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