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*Short Communication*

# Dopamine Partial Agonist Actions of the Glutamate Receptor Agonists LY 354,740 and LY 379,268

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*KEY WORDS* psychosis; glutamate agonist; dopamine agonist; LY 354,740; LY 379,268; D2 receptor; schizophrenia

*ABSTRACT* Because glutamate compounds alter the release of dopamine and prolactin, the present study examined whether group II metabotropic receptor agonists, LY 354,740 and LY 379,268, had any direct in vitro action on dopamine D2 receptors on rat striatal tissue, cloned D2Long receptors, and prolactin release from anterior pituitary cells. In competition versus the D2-specific ligand [$^3$H]domperidone, LY 354,740 had a dissociation constant of 24 nM at D2$^{High}$ (the functional high-affinity state of dopamine D2 receptors), while the value for LY 379,268 was 21 nM. LY 354,740 also stimulated by 50% the incorporation of [$^{35}$S]-GTP-γ-S at a concentration of 120 nM, but its maximal stimulation was only 22% of the maximum elicited by dopamine. LY 379,268 stimulated by 50% the incorporation of [$^{35}$S]-GTP-γ-S at 280 nM, but its maximal stimulation was also only 22% of the maximum elicited by dopamine. However, both LY 354,740 and LY 379,268 potently inhibited the dopamine-induced incorporation of [$^{35}$S]-GTP-γ-S with inhibitory Ki values of 43 nM and 30 nM, respectively. The release of prolactin from rat isolated anterior pituitary cells in culture was 50% inhibited by 20 nM LY 379,268 and by 100 nM LY 354,740. These Ki values are similar to those known for the mGluR II receptor, suggesting that these compounds may have both glutamate and dopamine actions in vivo. The dopamine agonist and antagonist actions of these compounds indicate that these drugs have properties of a dopamine partial agonist, and may, therefore, have antipsychotic action. **Synapse 62:154–158, 2008.** 

## INTRODUCTION

Although antipsychotics alleviate psychosis by inhibiting the action of dopamine on dopamine D2 receptors (reviewed by Seeman, 2006), the simultaneous antipsychotic stimulation of other receptors (e.g., serotonin-1A) helps to prevent side effects associated with D2 blockade (Bardin et al., 2006). In fact, it has long been proposed that the stimulation of glutamate receptors may also help to alleviate psychosis (reviewed by Goff and Coyle, 2001; Schoepp and Marek, 2002), but hitherto only moderate improvement has been found (Tuominen et al., 2005).

Moreover, because glutamate antagonists as well as agonists can increase the release of dopamine (Cartmell et al., 2000; Kegeles et al., 2000; Smith et al., 1998; van Berckel et al., 2006), it is essential to determine the receptor selectivity of these compounds. For example, it has been found that phencyclidine and ketamine have affinities for the high-affinity state of dopamine D2 receptors that are higher or similar to

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their affinities for the NMDA receptor (Kapur and Seeman, 2002; Seeman and Lasaga, 2005; Seeman et al., 2005; but see Jordan et al., 2006). In addition, the group II mGluR agonist L-CCG-1 (or [2S, 1′S, 2′S]-2-[carboxycyclopropyl]glycine) exerts a direct dopamine-like inhibition of prolactin release from cultured anterior pituitary cells (Caruso et al., 2004; Pampillo et al., 2002). Moreover, the group II mGluR agonist LY379,268 [or (−)-2-oxa-4-aminobicyclo[3.1.0]-hexane-4,6-dicarboxylate] was able to reduce hyperprolactinemia under several conditions (Johnson and Chamberlain, 2002). Therefore, because of the possibility that group II mGluR agonists such as LY 354,740 [or 2-amino-(1*S*,2*S*,5*R*,6*S*)-bicyclo{3.1.0} hexane-2,6-dicarboxylic acid monohydrate] and LY 379,268 might have a direct dopamine-like action or a dopamine partial agonist action at dopamine D2 receptors, the present study was designed to test these possibilities.

## MATERIALS AND METHODS

### Inhibition of [$^3$H]domperidone binding to dopamine D2 receptors

The potencies of LY 354,740 and LY 379,268 on the high-affinity state of the dopamine D2 receptor (or D2$^{High}$) were measured by competition with [$^3$H]domperidone, using rat striatal tissue and also human cloned dopamine D2Long receptors in CHO cells.

The striata were removed from rat brains (Sprague-Dawley) and stored at −70°C until used. The striata were homogenized in buffer (4 mg frozen tissue per ml buffer), using a teflon-glass homogenizer (with the piston rotating at 500 rpm) and 10 up and down strokes of the glass container. The buffer contained 50 mM Tris-HC1 (pH 7.4 at 20°C), 1 mM EDTA, 5 mM KCl, 1.5 mM $CaC1_2$, 4 mM $MgC1_2$, and 120 mM NaCl. The homogenate was not washed, centrifuged, or pre-incubated because previous work found that 30–50% of the D2 receptors were lost by these procedures (Seeman et al., 1984).

The dopamine D2 receptors in the rat striatum were measured with [$^3$H]domperidone (2 nM final concentration; custom synthesized as [phenyl-$^3$H(N)]domperidone; 68 Ci/mmol; PerkinElmer Life Sciences, Boston, MA; Seeman et al., 2003). Each incubation tube (12 mm × 75 mm, glass) received, in the following order, 0.5 ml buffer (with or without a final concentration of 200 μM GN [guanilylimidodiphosphate]), and with or without a final concentration of 10 μM S-sulpiride (to define nonspecific binding to the dopamine D2 receptors), 0.25 ml [$^3$H]domperidone, and 0.25 ml of tissue homogenate. The tubes, containing a total volume of 1 ml, were incubated for 2 h at room temperature (20°C), after which the incubates were filtered, using a 12-well cell harvester (Titertek, Skatron, Lier, Norway) and buffer-presoaked glass fiber filter mats (Whatman GF/C). After filtering the incubate the filter mat was rinsed with buffer for 15 s (7.5 ml buffer). The filters were pushed out and placed in scintillation minivials (7 ml, 16 mm × 54 mm; Valley Container, Bridgeport, CO). The minivials received 4 ml each of scintillant (Research Products International Corp., Mount Prospect, IL), and were monitored 6 h later for tritium in a Beckman L5000 scintillation spectrometer at 55% efficiency. The specific binding of [$^3$H]domperidone was defined as total binding minus that in the presence of 10 μM S-sulpiride.

Human cloned dopamine D2Long receptors, expressed in Chinese Hamster Ovary (CHO) cells, were used, as previously described (Liu et al., 2000), and the tissue processed by a procedure similar to that described above for the rat striatal tissue.

### Incorporation of [$^{35}$S]GTP-γ-S into CHO cells containing dopamine D2 receptors

The effect of dopamine, LY 354,740, or LY 379,268 on the incorporation of [$^{35}$S]GTP-γ-S was measured as previously described (Kapur and Seeman, 2002; Seeman et al., 2005). Human cloned D2Long receptors were expressed in CHO cells as described earlier. The homogenized tissue was suspended in assay buffer (50 mM Tris, pH 7.4, 1 mM EDTA, 5 mM KCl, 4 mM $MgCl_2$, 1.5 mM $CaCl_2$, 120 mM NaCl, and 10 μM GDP). The final incubation glass test tube (12 mm × 75 mm) received 0.25 ml of dopamine and/or LY354,740 and/or LY 379,268, 0.25 ml of [$^{35}$S]GTP-γ-S (1,250 Ci/mmol, final concentration of 0.2 nM; Perkin-Elmer Life Sciences, Boston, MA), and 0.5 ml of cell suspension. The reaction mixture was incubated for 30 min in a 30°C water bath. The reaction was terminated by rapid filtration and the radioactivity measured by liquid scintillation spectrometry, as noted earlier.

### Release of prolactin

The effect of LY 379,268 and LY 354,740 on the release of prolactin from rat isolated anterior pituitary cells in primary tissue culture was measured as previously reported (Seeman and Lasaga, 2005), except that the final measurement of rat prolactin was done by means of rat prolactin ELISA kits obtained from MD Biosciences (St. Paul, MN) and using the procedure recommended by the manufacturer. The anterior pituitary culture has 45–50% lactotrophs, 20% somatotropes, with the remainder being tyrotropes, corticotropes, and gonadotropes.

## RESULTS

The representative experiments in Figure 1 show that LY 354,740 (Fig. 1, top) and LY 379,268 (Fig. 1, bottom) inhibited the binding of [$^3$H]domperidone in a biphasic manner typical of a dopamine agonist

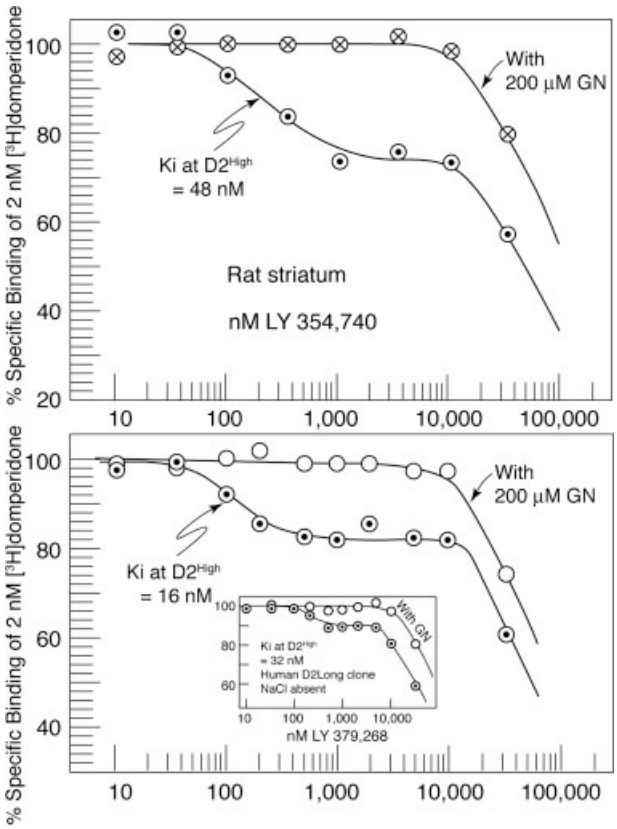


Fig. 1. Representative experiments for the biphasic inhibition by LY 354,740 (top) and by LY 379,268 (bottom) of the binding of 2 nM [$^3$H]domperidone to homogenized rat striatal tissue (inset shows data for D2 clone). In these representative experiments, the Ki$^{High}$ for the high-affinity phase was 48 nM for LY 354,740 (top), and was 16 nM for LY 379,268 (bottom). The high-affinity phase was entirely abolished by the presence of 200 μM guanilylimidodiphosphate (or GN), indicating the agonist nature of LY 354,740 (top). The Ki for the low-affinity component was 18,000 nM for LY 354,740. The Ki for LY 379,268 at D2$^{High}$ in the human D2Long clone was 32 nM; this was done in the absence of NaCl in order to reveal the high-affinity state of D2 more readily. Nonspecific binding was defined by the presence of 1 μM S-sulpiride.

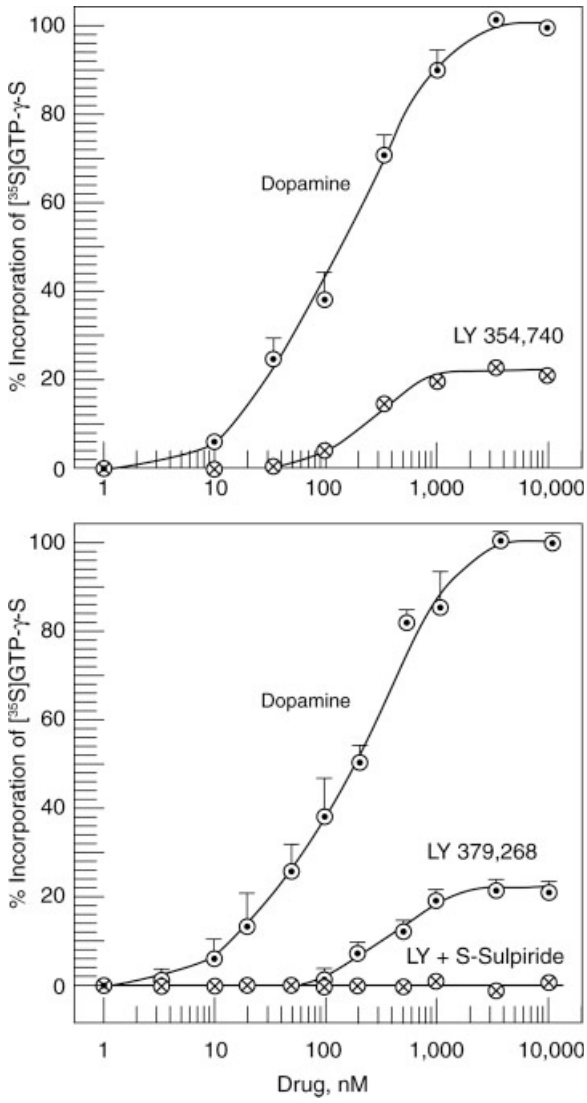


Fig. 2. Stimulation by dopamine, LY 354,740 (top) and LY 379,268 (bottom) of the incorporation of [$^{35}$S]-GTP-γ-S into CHO cells containing cloned dopamine D2Long receptors. Although both LY compounds stimulated the incorporation, they did so at a level of only 22% of that stimulated by dopamine. Final concentration of [$^{35}$S]-GTP-γ-S was 0.2 nM. The error bars indicate S.E. ($n$ = 4 independent experiments). The stimulating action of these compounds was fully inhibited by 10 μM S-sulpiride (bottom).

(Seeman et al., 2003, 2005). In the experiments shown (Fig. 1) LY 354,740 and LY 379,268 inhibited 50% of the binding of [$^3$H]domperidone at the high-affinity component with dissociation constants, Ki$^{High}$, of 48 nM and 27 nM, respectively. Each dissociation constant, Ki$^{High}$, was derived from the commonly used Cheng-Prusoff equation, IC50/(1 + $C^*/K_d$), where IC50 was the concentration that inhibited the binding at either the high- or low-affinity component by 50%, where $C^*$ was the final molarity of [$^3$H]domperidone (2 nM), and where $K_d$ was the dissociation constant for [$^3$H]domperidone (0.38 nM). In a series of four such experiments, the average Ki$^{High}$ for LY 354,740 at the D2$^{High}$ site was 24 ± 9 nM, while that for LY 379,268 was 21.1 ± 3.4 nM ($n$ = 4).

The agonist actions of dopamine, LY 354,740 and LY 379,268 on the incorporation of [$^{35}$S]-GTP-γ-S are shown in Figure 2. The maximum levels of stimulation or intrinsic efficacies of LY 354,740 and LY 379,268 were both only 22% of that elicited by dopamine.

However, despite the relatively low levels of D2-stimulating efficacies of LY 354,740 and LY 379,268, the compounds fully inhibited the D2-stimulating action of 1 μM dopamine by inhibiting 50% of the dopamine-induced incorporation of [$^{35}$S]-GTP-γ-S at 400 nM and 120 nM, respectively (Fig. 3; $N$ = 4). Hence, the Ki values for LY 354,740 and LY 379,268 to inhibit the D2-stimulating action of dopamine may be derived by Ki = C50%/(1 + $D$/EC50%), where C50% was the concentration of LY 354,740 or LY 379,268 that 50% inhibited the effect of 1 μM dopamine

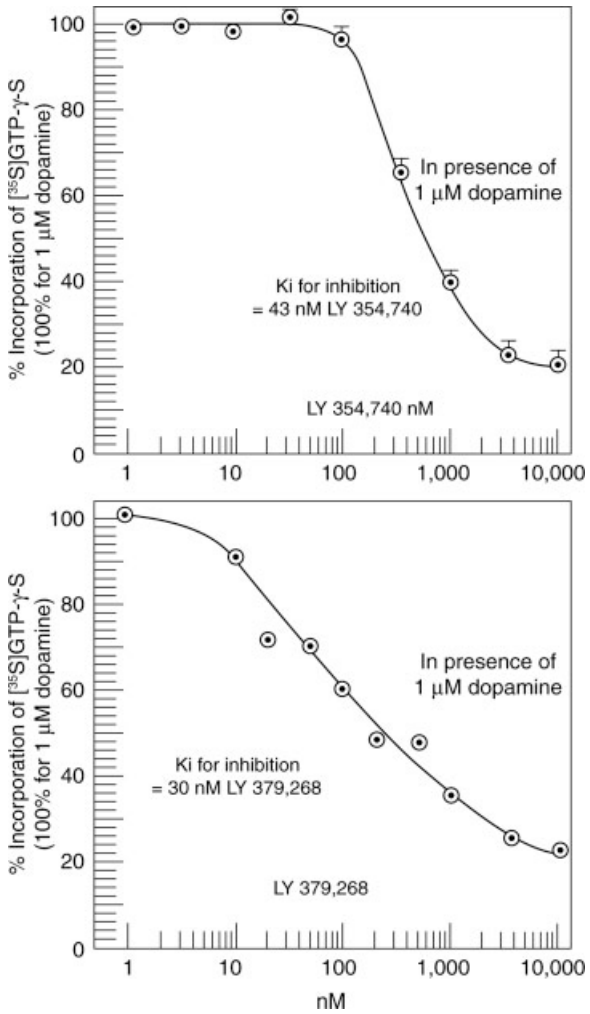


Fig. 3. The stimulation of [$^{35}$S]-GTP-γ-S incorporation by 1 μM dopamine was 50% inhibited by 400 nM LY 354,740 (yielding an inhibitory Ki value of 43 nM) and by 280 nM LY 379,268 (yielding an average inhibitory Ki value of 27 nM). Final concentration of [$^{35}$S]-GTP-γ-S was 0.2 nM. The error bars (top) indicate S.E. ($n$ = 4 independent experiments). The experiment at the bottom was representative of three independent experiments.

(namely, 400 nM or 120 nM), where $D$ was the concentration of dopamine (1000 nM), and where EC50% was the concentration of dopamine that 50% stimulated the incorporation of [$^{35}$S]-GTP-γ-S (i.e. 120 nM, Fig. 2). Therefore, using the appropriate values, the Ki for LY 354,740 to inhibit the action of dopamine on D2 was 43 nM, while that for LY 379,268 was 27 nM.

Finally, in order to determine whether these two LY compounds acted on D2$^{High}$, LY 354,740 and LY 379,268 were tested on the release of prolactin from cultured anterior pituitary cells isolated from the rat. The release of prolactin was approximately 50% inhibited at 20 nM LY 379,268, and 100 nM LY 354,740, as shown in Figure 4. The maximum inhibition of prolactin release occurred at 100 nM for LY 379,268 and at 1000 nM for LY 354,740.

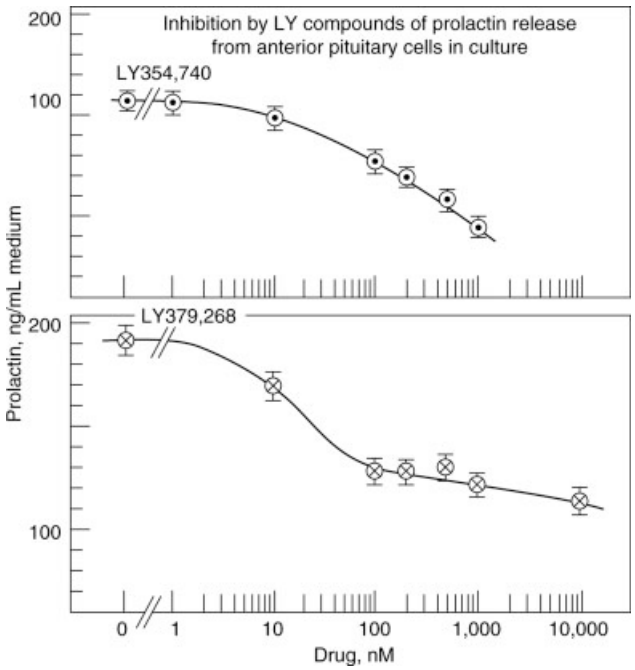


Fig. 4. The release of prolactin from lactotropes within the rat isolated anterior pituitary cells in culture was inhibited by LY354,740 (top) and by LY 379,268 (bottom) after 4 h incubation. Given that the maximum inhibition occurred at 1000 nM for both drugs, the concentration for 50% inhibition was 20 nM for LY 379,268 and 100 nM for LY 354,740.

## DISCUSSION

Although the maximum D2-stimulating actions of LY 354,740 and LY 379,268 were only 22% of that for dopamine (Fig. 2), both drugs effectively inhibited the action of dopamine with inhibitory Ki values of 43 nM and 27 nM, respectively (Fig. 3). In addition, the two compounds effectively stimulated D2$^{High}$ (with IC50% values of 100 nM and 20 nM, respectively) to inhibit prolactin release (Fig. 4).

The value of 43 nM for the inhibitory Ki value of LY 354,740 was similar to its Ki$^{High}$ value of 24 nM for the inhibition of the binding of [$^{3}$H]domperidone to D2$^{High}$ (Fig. 1). In addition, the value of 27 nM for the inhibitory Ki value of LY 3379,268 was similar to its Ki$^{High}$ value of 21 nM for the inhibition of the binding of [$^{3}$H]domperidone to D2$^{High}$ (Fig. 1).

These Ki values of 24 and 48 nM for LY 354,740 at D2$^{High}$ are similar to the LY 354,740 Ki values of 16, 75, and 114 nM at the mGluR2 receptor, as summarized and reviewed by Schoepp et al. (1999).

Moreover, the Ki value of 21 nM for LY 379,268 at D2$^{High}$ is similar to the LY 379,268 Ki values of 14 and 15 nM at the mGluR2 receptor (rat brain and cloned mGlu2, respectively), using the ligand [$^{3}$H]LY 341,495, as summarized and reviewed by Schoepp et al. (1999).

It is likely, therefore, that the actions of LY 354,740 and LY 379,268 in vivo (Cartmell et al., 2000;

Schoepp et al., 1998) have both glutamate and dopamine components of action. For example, the enhancement by LY 354,740 of amphetamine-induced release of dopamine (van Berckel et al., 2006) may result not only from mGluR2 stimulation but also from presynaptic D2 receptor inhibition by LY 354,740. A similar situation may apply for the ketamine-induced release of dopamine and for ketamine's enhancement of amphetamine-induced release of dopamine (Kegeles et al., 2000; Smith et al., 1998).

Moreover, although LY 379,268 inhibits the release of glutamate in the brain, it also inhibits the release of dopamine (Greenslade and Mitchell, 2004), and may be acting as a pre-synaptic dopamine agonist on dopamine autoreceptors to inhibit dopamine release.

The D2-stimulating actions of LY 354,740 and LY 379,268 and their associated inhibition of the stimulating action of dopamine on D2 in vitro is similar to the dopamine-inhibiting action found by Cosi et al. (2006) for the partial agonist aripiprazole, an effective antipsychotic. This similarity warrants referring to LY 354,740 and LY 379,268 as dopamine partial agonists, Furthermore, their dopamine partial agonist action, and, therefore, their dopamine effectively inhibitory action, is compatible with an expected antipsychotic clinical effect of closely related congeners (Patil et al., 2007). The dopamine agonist-like properties of these compounds, however, are best examined not by using [$^3$H]raclopride or [$^3$H]spiperone, which do not readily reveal the D2$^{High}$ state (Seeman et al., 2003), but by competition with [$^3$H]domperidone, as described in the present study.

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