Research report

Dopamine and 5-HT turnover are increased by the mGlu2/3 receptor agonist LY379268 in rat medial prefrontal cortex, nucleus accumbens and striatum

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Abstract

We have shown, using in vivo microdialysis sampling, that systemic administration of the selective group II metabotropic (mGlu) receptor agonist LY379268, like the atypical antipsychotic clozapine, increased extracellular levels of dopamine, dopamine metabolites DOPAC and HVA, and the major 5-HT metabolite 5-HIAA, in rat medial prefrontal cortex (mPFC). Here, we have compared the effects of LY379268 with clozapine as well as risperidone on ex vivo tissue levels of dopamine, DOPAC, HVA, 5-HT and 5-HIAA in multiple brain regions. One to two hours following administration of LY379268, mPFC tissue levels of DOPAC, HVA and 5-HIAA were increased in a dose-dependent manner. Increases evoked by LY379268 (10 mg/kg s.c.) at the 2 h point were 189, 245 and 139% of basal levels, respectively. These effects were reversed within 4 h of administration. Clozapine (10 mg/kg s.c.) and risperidone (1 mg/kg s.c.) also increased levels of the dopamine metabolites to a similar extent but were without significant effect on tissue levels of 5-HIAA. LY379268 (10 mg/kg s.c.) also increased tissue levels of DOPAC, HVA and 5-HIAA by 169, 221 and 134% of basal levels in nucleus accumbens, respectively, and by 131, 179 and 132% of basal levels in striatum, respectively. These data show that activation of mGlu2/3 receptors can increase the turnover of dopamine and 5-HT in the areas of the brain implicated in the actions of atypical antipsychotics. © 2000 Elsevier Science B.V. All rights reserved.

Theme: Neurotransmitters, modulators, transporters, and receptors

Topic: Excitatory amino acids: physiology, pharmacology and modulation

Keywords: Metabotropic glutamate receptor; Dopamine; 5-HT; Prefrontal cortex; Nucleus accumbens; Striatum; Turnover

1. Introduction

Metabotropic glutamate (mGlu) receptors are a heterogeneous family of G-protein coupled receptors that function to modulate glutamate and non-glutamate neuronal transmission by presynaptic, postsynaptic and glial mechanisms [4,16]. Our earlier studies demonstrated that systemic injection of the potent, selective group II metabotropic glutamate (mGlu2/3) receptor agonist, LY379268 [14] increased extracellular levels of dopamine, dopamine metabolites DOPAC and HVA, and the major 5-HT metabolite 5-HIAA in the medial prefrontal cortex (mPFC) of the freely-moving rat [3]. In eliciting these effects on monoamines and their metabolites in the mPFC, LY379268 shares a similar profile to the atypical antipsychotics, clozapine and risperidone [3,6,7]. In addition to increasing extracellular levels of dopamine, clozapine and the classical neuroleptic haloperidol, also increase brain tissue levels of the dopamine metabolites 3,4-dihydroyphenylacetic acid (DOPAC) and homovanillic acid (HVA) [10,13,19,20]. Specifically, Karoum and Egan [10] showed that clozapine increased ex vivo tissue levels of DOPAC in three of the brain regions that have been implicated in the actions of antipsychotics: PFC, nucleus accumbens and the striatum. Clozapine also increased HVA levels in the striatum and nucleus accumbens [19].

Although metabolite tissue levels do not necessarily correlate directly with neurotransmitter release, many
studies have indicated that levels of the dopamine metabolites DOPAC and HVA in tissue are useful as indexes of neurotransmitter function [15,18]. In particular, the tissue metabolite:neurotransmitter ratios such as DOPAC:dopamine ratio have been calculated to study drug-induced changes neurotransmitter utilization across different brain regions [8,9].

Given the similarities between the effects of LY379268 and atypical antipsychotics on monoamine levels in the extracellular fluid of the mPFC [3], along with earlier observation that LY379268 acts like clozapine in animal behavioral tests of psychosis [2], here we have examined the effects of LY379268 on ex vivo tissue levels of dopamine, 5-HT and their metabolites DOPAC and HVA, and 5-hydroxyindoleacetic acid (5-HIAA). By calculating the tissue DOPAC:dopamine, HVA:dopamine and 5-HIAA:5-HT ratios, we were able to obtain an indication of dopamine and 5-HT turnover/utilization in tissue regions of the rat brain. We have also compared the effects of LY379268 in the medial prefrontal cortex with two different atypical antipsychotics, clozapine and risperidone. Furthermore, we studied the actions of LY379268 (10 mg/kg s.c.) in two other brain regions, the nucleus accumbens and striatum. Overall, these studies are aimed at further understanding the possible role of mGlu2/3 receptor activation in psychiatric disorders.

2. Materials and methods

Experiments were essentially performed as described by Fuller and Perry [5] and Bymaster et al. [1] with some modifications.

2.1. Tissue preparation

Male Sprague–Dawley rats (310–325 g) were group-housed (maximum of six rats per cage) under standard laboratory conditions with ad libitum access to food and water (12 h light–dark cycle). Rats were removed from their home cages and injected s.c. with LY379268, clozapine, risperidone or sterile water s.c. and then replaced in their cages. At the required time following the injection (0.5, 1, 2 or 4 h postinjection for the time course study; 2 h postinjection for the other studies), rats were removed from the cage and killed via decapitation in an adjacent room. The prefrontal cortex, nucleus accumbens and/or striatum were dissected out and frozen on dry ice. The tissue samples were then weighed individually and stored at −80°C in plastic tubes containing 0.5 ml of 0.01 M HCl until analyzed for DOPAC, HVA, dopamine, 5-HIAA and 5-HT. Immediately before analysis, samples were thawed at room temperature and 0.4 ml of 0.01 M HCl (containing isoproterenol as an internal standard) was added. After sonication, 100 μl of 1.5 M perchloric acid was added and then the samples were vortexed and stored at 4°C for 30 min. The samples were then centrifuged in a bench top centrifuge (2 min at 12,000 rpm) and the supernatant analyzed by HPLC with electrochemical detection.

2.2. HPLC analysis

A BDS Hypersil C18 column (100×4.6 mm) from Keystone Scientific (Bellefonte, PA, USA) with a 100-μl loop was used to analyze the supernatant (injection volume 50 μl) for levels of dopamine, DOPAC, HVA, 5-HT and 5-HIAA. The mobile phase consisted of 75 mM sodium phosphate monobasic, 350 mg/l octanesulfonic acid sodium salt, 0.5 mM EDTA, 1% tetrahydrofuran (HPLC grade, inhibitor-free) and 9% acetonitrile at pH 3 (adjusted with phosphoric acid). The analytical column, with flow-rate 1 ml/min, was maintained at 40°C with a column heater. An electrochemical detector (Princeton Applied Research, Oak Ridge, TN, USA) with dual glassy carbon electrodes was used (E1 = 650 mV, E2 = 65 mV). Ranges were 1 nA for detection of 5-HIAA and 5-HT at E1, and 0.5 nA for detection of DOPAC, HVA and dopamine at E2 for mPFC samples. Ranges were 100 nA and 20 nA for E1 and E2, respectively for nucleus accumbens and striatum samples. The data from both channels were collected by EZCHROM ELITE software, which calculates peak heights and sample concentrations.

2.3. Materials

Clozapine and risperidone were purchased from Research Biochemicals (Natick, MA, USA). LY379268 was synthesized by James A. Monn at Lilly Research Laboratories (Indianapolis, USA) [14].

2.4. Data analysis

Statistical analyses were carried out using the GRAPHPAD PRISM graphics program. Data were evaluated by a one-way analysis of variance (ANOVA) and posthoc comparisons were conducted using the Neuman–Keuls test for multiple comparisons. Statistical significance in the Neuman–Keuls test is shown by asterisks with a criterion of $P < 0.05$ considered to be statistically significant (Tables 1 and 2, Figs. 1 and 2). Data from experiments determining the effects in different brain areas were evaluated using an unpaired t test and statistical significance ($P < 0.05$) in this test is also shown by asterisks (Table 3 and Fig. 3).

3. Results

3.1. Time course of LY379268 effect in mPFC

LY379268 (10 mg/kg s.c.) increased dopamine and 5-HT turnover in the mPFC as shown by the ratios of
DOPAC: dopamine (Fig. 1A), HVA: dopamine (Fig. 1B) and 5-HIAA: 5-HT (Fig. 1C). These increases were time-dependent, the ratios were maximal 2 h following administration of LY379268 (the HVA: dopamine ratio was statistically significant at this time only) and the increases were abolished at the four h time point (Fig. 1).

Table 1 shows that absolute levels of DOPAC were increased at 0.5, 1 and 2 h after administration of LY379268 (10 mg/kg s.c.) by 170, 196 and 189% of basal levels, respectively (i.e. compared with rats treated with vehicle). HVA was also significantly increased at the 1 and 2 h time points by 186 and 245% of basal levels, respectively. In this experiment, LY379268 (10 mg/kg s.c.) also maximally increased tissue levels of dopamine to 147% of basal levels 1 h after administration, but a statistically significant increase in dopamine turnover was not observed in other experiments (see Tables 2 and 3). 5-HT levels per se were not significantly different in rats treated with LY379268 and those rats given vehicle (Table 1). However, tissue levels of 5-HIAA were augmented by LY379268 (10 mg/kg s.c.) in a time-dependent manner, increases were 124 and 139% of basal levels at the 1 and 2 h time points, respectively.

3.2. Comparison of LY379268 with clozapine and risperidone in mPFC

The increases in dopamine and 5-HT turnover evoked by LY379268 were dose-dependent (Fig. 2). The DOPAC: dopamine ratio was significantly increased at doses of 3 and 10 mg/kg (but not 1 mg/kg) LY379268 and by 10 mg/kg clozapine and 1 mg/kg risperidone (Fig. 2A). In this experiment the effects of LY379268 (up to 10 mg/kg s.c.) and clozapine (10 mg/kg s.c.) on the HVA: dopamine ratio were not statistically significant probably due to the larger standard error in HVA levels in the mPFC (Table 2). However, risperidone (1 mg/kg s.c.) did produce a significant increase in the HVA: dopamine ratio (Fig. 2B).

As shown in Table 2, absolute levels of DOPAC were increased by 3 and 10 mg/kg LY379268 (146 and 174% of basal levels) and particularly by 1 mg/kg risperidone (189% of basal levels). Clozapine (10 mg/kg s.c.) did produce a 137% increase in DOPAC levels but, in this experiment, the effect was not statistically significant. Risperidone also significantly increased HVA levels by 170% of basal, and although 10 mg/kg LY379268 increased HVA to 160% and 10 mg/kg clozapine to 144% of basal, these effects were not statistically significant probably due to variability in the HVA values.

All three doses of LY379268 tested (from 1 to 10 mg/kg s.c.) increased the 5-HIAA: 5-HT ratio (Fig. 2C). 5-HT turnover was also increased by risperidone (1 mg/kg s.c.) and clozapine (10 mg/kg s.c.). However, although there was a statistically significant increase in the 5-HIAA: 5-HT ratio (Fig. 2C), it should be noted that 10 mg/kg clozapine had no effect per se on mPFC tissue levels of 5-HIAA (Table 2). The apparent increase in turnover actually reflects the statistically significant reduction (17% inhibition) in 5-HT levels evoked by 10 mg/kg clozapine (Table 2). Although clozapine (10 mg/kg s.c.) and risperidone (1 mg/kg s.c.) had no statistically significant effects on 5-HIAA levels (Table 2), LY379268 (at 3

Table 1
Time course of the effects of LY379268 (10 mg/kg s.c.) on tissue levels of dopamine, 5-HT and their respective metabolites in the medial prefrontal cortex.

<table>
<thead>
<tr>
<th>Time</th>
<th>DOPAC</th>
<th>HVA</th>
<th>DA</th>
<th>5-HIAA</th>
<th>5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.128±0.013</td>
<td>0.112±0.015</td>
<td>0.281±0.030</td>
<td>1.709±0.085</td>
<td>1.894±0.097</td>
</tr>
<tr>
<td>0.5 h</td>
<td>0.218±0.014*</td>
<td>0.159±0.016</td>
<td>0.357±0.030</td>
<td>1.902±0.090</td>
<td>1.750±0.100</td>
</tr>
<tr>
<td>1 h</td>
<td>0.251±0.010*</td>
<td>0.208±0.019*</td>
<td>0.412±0.015*</td>
<td>2.114±0.078*</td>
<td>1.976±0.048</td>
</tr>
<tr>
<td>2 h</td>
<td>0.242±0.015*</td>
<td>0.272±0.025*</td>
<td>0.384±0.027*</td>
<td>2.375±0.091*</td>
<td>1.987±0.077</td>
</tr>
<tr>
<td>4 h</td>
<td>0.151±0.013</td>
<td>0.172±0.014</td>
<td>0.319±0.024</td>
<td>1.975±0.152</td>
<td>1.961±0.111</td>
</tr>
</tbody>
</table>

* Data are presented as mean±S.E.M. levels (nmol/g tissue), n = 8 rats. *, P<0.05 when compared with the corresponding control in vehicle treated rats using the Neuman–Keuls multiple comparison test.

Table 2
Effects of LY379268 (1, 3 and 10 mg/kg s.c.), clozapine (10 mg/kg s.c.) and risperidone (1 mg/kg s.c.) on tissue levels of dopamine, 5-HT and their respective metabolites in the medial prefrontal cortex.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DOPAC</th>
<th>HVA</th>
<th>DA</th>
<th>5-HIAA</th>
<th>5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.090±0.007</td>
<td>0.144±0.012</td>
<td>0.343±0.022</td>
<td>1.108±0.049</td>
<td>2.185±0.124</td>
</tr>
<tr>
<td>LY379268 1 mg/kg</td>
<td>0.096±0.007</td>
<td>0.145±0.017</td>
<td>0.286±0.015</td>
<td>1.201±0.059</td>
<td>1.921±0.045</td>
</tr>
<tr>
<td>LY379268 3 mg/kg</td>
<td>0.131±0.011*</td>
<td>0.191±0.028</td>
<td>0.351±0.017</td>
<td>1.346±0.051*</td>
<td>2.008±0.055</td>
</tr>
<tr>
<td>LY379268 10 mg/kg</td>
<td>0.157±0.009*</td>
<td>0.230±0.027</td>
<td>0.394±0.015</td>
<td>1.524±0.068*</td>
<td>2.128±0.118</td>
</tr>
<tr>
<td>Clozapine 10 mg/kg</td>
<td>0.123±0.016</td>
<td>0.207±0.032</td>
<td>0.344±0.043</td>
<td>1.147±0.084</td>
<td>1.805±0.128*</td>
</tr>
<tr>
<td>Risperidone 1 mg/kg</td>
<td>0.170±0.011*</td>
<td>0.245±0.027*</td>
<td>0.330±0.024</td>
<td>1.290±0.046</td>
<td>2.003±0.059</td>
</tr>
</tbody>
</table>

* Data are presented as mean±S.E.M. levels (nmol/g tissue), n = 7 rats. *, P<0.05 when compared with the corresponding control in vehicle treated rats using the Neuman–Keuls multiple comparison test.
Fig. 1. Time course for LY379268 (10 mg/kg s.c.)-evoked increases in the turnover of dopamine and 5-HT in the rat mPFC. Data are expressed as the mean±S.E.M. (A) DOPAC:dopamine (B) HVA:dopamine and (C) 5-HIAA:5-HT ratios; n=8 rats. Actual levels of DOPAC, HVA, dopamine, 5-HIAA and 5-HT are shown in Table 1. *, P<0.05 when compared with the corresponding control in vehicle treated rats using the Neuman–Keuls multiple comparison test.

Fig. 2. LY379268 dose–response for increases turnover of dopamine and 5-HT in the mPFC, and its comparison with atypical antipsychotics. Rats were killed 2 h postadministration of drug vehicle (VEH), LY379268 (1, 3 and 10 mg/kg, s.c.), clozapine (10 mg/kg s.c.) and risperidone (1 mg/kg s.c.). Data are expressed as the mean±S.E.M. (A) DOPAC:dopamine (B) HVA:dopamine and (C) 5-HIAA:5-HT ratios; n=7 rats. Actual levels of DOPAC, HVA, dopamine, 5-HIAA and 5-HT are shown in Table 2. *, P<0.05 when compared with the corresponding control in vehicle treated rats using the Neuman–Keuls multiple comparison test.
and 10 mg/kg s.c.) did increase absolute levels of 5-HIAA (by 121 and 138% of basal levels respectively). None of the drugs tested increased 5-HT levels in mPFC tissue (Table 2).

3.3. Effect of LY379268 in nucleus accumbens and striatum

Dopamine turnover as measured by DOPAC:dopamine and HVA:dopamine ratios was increased by 10 mg/kg LY379268 s.c. in the nucleus accumbens and striatum as well as the mPFC (Fig. 3A and B). In addition, turnover of 5-HT was also significantly increased, as reflected by the 5-HIAA:5-HT ratio, in all three brain areas (Fig. 3C).

Furthermore, absolute tissue levels of DOPAC, HVA and 5-HIAA were all significantly increased by 10 mg/kg LY379268 s.c. in the mPFC, nucleus accumbens and striatum (Table 3). DOPAC levels were increased by 145, 169 and 131% of basal levels in the mPFC, nucleus accumbens and striatum, respectively. Increases in HVA were 191, 221 and 179%, and increases in 5-HIAA were 128, 134 and 132% of basal levels in the mPFC, nucleus accumbens and striatum, respectively. Dopamine levels were not affected in any of the three areas by 10 mg/kg LY379268. In addition, although there was a slight, but statistically significant, increase (112% of basal levels) in the nucleus accumbens, 10 mg/kg LY379268 did not affect 5-HT levels in the mPFC or striatum.

4. Discussion

In earlier studies using in vivo microdialysis we have demonstrated that systemic administration of the selective mGlu2/3 receptor agonist LY379268 evoked similar effects on the monoaminergic systems of the mPFC as the atypical antipsychotic clozapine [3]. In this paper, we have compared the effects of LY379268 with two different atypical antipsychotics, clozapine and risperidone, on dopamine and 5-HT turnover in ex vivo mPFC tissue.

There are a number of methods to measure monoamine neurotransmitter turnover/utilization in the brain. Here we used the metabolite to neurotransmitter ratios as a way to reflect neurotransmitter utilization in order to compare LY379268 to other mechanistically distinct clinically effective antipsychotic compounds which are well documented to increase monoamine turnover by this method. The LY379268 data here, along with our previous microdialysis study [3] showing enhanced dopamine release, strongly suggests that changes in tissue metabolite to neurotransmitter ratios induced by LY379268 represent changes in monamine turnover/utilization. The effects evoked by clozapine on dopamine turnover in mPFC tissue in this study were similar to those published by others. Karoum and Egan [10] showed that systemic injection of 10 mg/kg clozapine increased DOPAC tissue levels in the frontal cortex by 138% of basal (we observed an increase of 137%). In addition, clozapine (10 mg/kg) evoked similar increases in tissue DOPAC and HVA levels in the nucleus accumbens and striatum [10,17,19].

Despite sampling from different neurotransmitter pools, there are similarities between the results of these tissue level studies and in vivo microdialysis studies. Effective doses of LY379268 (1–10 mg/kg s.c.), clozapine (10 mg/kg s.c.) and risperidone (1 mg/kg s.c.) which increased tissue levels of DOPAC, HVA and 5-HIAA are comparable with previous microdialysis studies [3,6,10]. Also, the time of onset of these effects were similar, although in the microdialysis tests levels of metabolites remained elevated up to 4 h following injection of LY379268, whereas in the current tissue study levels were not significantly different from controls at this time point.

The LY379268-evoked increases in metabolites were dose-dependent with a ~ED50 for increases in absolute levels of the metabolites around 1–3 mg/kg LY379268. Both clozapine (10 mg/kg) and risperidone (1 mg/kg) mimicked the effects of LY379268 on mPFC DOPAC and HVA levels. However, increases in 5-HIAA were evoked only by LY379268 and risperidone (clozapine had no significant effect on 5-HIAA and decreased 5-HT levels). Interestingly, the ~ED50 doses of LY379268 which enhance monoamine release and turnover (1–3 mg/kg s.c.), are those which we have shown in earlier work to block certain phencyclidine and amphetamine behaviors in rats.

Table 3

<table>
<thead>
<tr>
<th>Area</th>
<th>Condition</th>
<th>DOPAC</th>
<th>HVA</th>
<th>DA</th>
<th>5-HIAA</th>
<th>5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>mPFC</td>
<td>Vehicle</td>
<td>0.11±0.01</td>
<td>0.11±0.02</td>
<td>0.37±0.02</td>
<td>1.40±0.08</td>
<td>2.02±0.09</td>
</tr>
<tr>
<td></td>
<td>LY379268 10 mg/kg</td>
<td>0.16±0.01*</td>
<td>0.21±0.02*</td>
<td>0.44±0.02</td>
<td>1.79±0.06*</td>
<td>2.09±0.09</td>
</tr>
<tr>
<td>Nuc. acc.</td>
<td>Vehicle</td>
<td>9.88±0.52</td>
<td>4.25±0.29</td>
<td>60.66±2.07</td>
<td>5.80±0.27</td>
<td>7.34±0.33</td>
</tr>
<tr>
<td></td>
<td>LY379268 10 mg/kg</td>
<td>16.72±0.68*</td>
<td>9.38±0.61*</td>
<td>67.40±3.30</td>
<td>7.76±0.18*</td>
<td>8.19±0.15*</td>
</tr>
<tr>
<td>Striatum</td>
<td>Vehicle</td>
<td>12.54±0.87</td>
<td>5.84±0.26</td>
<td>96.77±5.30</td>
<td>6.10±0.20</td>
<td>4.20±0.19</td>
</tr>
<tr>
<td></td>
<td>LY379268 10 mg/kg</td>
<td>16.47±1.21*</td>
<td>10.44±0.98*</td>
<td>96.87±9.62</td>
<td>8.05±0.25*</td>
<td>4.35±0.17</td>
</tr>
</tbody>
</table>

*Data are presented as mean±S.E.M. levels (nmol/g tissue), n=8 rats. *, P<0.05 when compared with the corresponding control in vehicle treated rats using an unpaired t test.
Nevertheless, the relevance of these neurochemical effects of LY379268 to its actions in animal tests of psychosis are not yet clear, as psychotomimetic agents such phencyclidine, like atypical antipsychotics and LY379268, will also increase dopamine release and turnover [8,9]. Likewise, non-competitive NMDA receptor antagonists have also been reported to increase extracellular 5-HT levels [12], possibly by increasing the firing rate of raphe neurons [11].

In summary, these data extend the results of our previous in vivo microdialysis studies, as the activation of mGlu2/3 receptors by LY379268 increased the tissue turnover/utilization of dopamine and 5-HT in the mPFC as well as other areas of the brain implicated in the mechanisms of action of atypical antipsychotics. Additional work is needed to address the relevant cellular and synaptic mechanisms responsible for these actions of the mGlu2/3 agonists and their possible therapeutic significance.

References


