Elucidation of D₃ receptor function in vivo: do D₃ receptors mediate inhibition of dopamine neuronal activity?

C. Routledge, L. Thorn, T. Ashmeade and S. Taylor
Psychiatry Research Department, SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, U.K.

A number of studies have recently suggested that the D₃ receptor [1] is located presynaptically and functions as an autoreceptor [2-4]. These studies have demonstrated that 7-hydroxy-di-n-propyl-2-aminoetatralin [(+)-7-OH-DPAT], a ligand with significant D₃ receptor affinity, decreases dopamine release in limbic and striatal regions. However, the selectivity of (+)-7-OH-DPAT for the D₃ receptor is based on binding data [1,2]. When characterized functionally, there is little difference (four- to seven-fold) in the efficacy of (+)-7-OH-DPAT at the D₃ and D₂ receptor [5,6]. This calls into question the claims that certain in vivo responses, which have been characterized using (+)-7-OH-DPAT, are mediated via D₃ receptors. It also suggests that (+)-7-OH-DPAT may not be the ligand of choice for elucidating D₃ receptor function in vivo. The discrepancy between binding and functional values for D₃ receptor ligands may, however, be a consequence of using recombinant systems; it remains to be determined if this difference is extrapolated from cloned human receptors expressed in artificial cell lines to native animal tissue.

Whether using functional or binding data, one of the most selective D₃ ligands to date is quinpirole (LY-163502). This compound has at least 100-fold greater affinity for the D₃ than for the D₂ receptor. Functionally (stimulation of mitogenesis), this compound is over 20-fold more selective for the D₃ receptor [6]. In the present study we have characterized the profile for quinpirole plus a number of other D₃ and D₃/D₂ receptor ligands on dopamine release and synthesis in the rat nucleus accumbens (n. accumbens) and striatum (the n. accumbens has a higher D₃/D₂ receptor density than the striatum) in attempts to characterize further D₃ receptor function in vivo.

For synthesis studies, male CD1 mice (Charles River) were administered varying concentrations of apomorphine, 7-OH-DPAT, (+)PHNO [(+)-4-propyl-9-hydroxynaphthoxazine-HCl], quinpirole (LY-163502) and (-)quinpirole (LY-171555) followed 5 min later by administration of γ-hydroxybutyrolactone (GBL, 750 mg/kg intraperitoneally) or vehicle (0.9% NaCl, 0.1 ml/kg intraperitoneally). After an additional 5 min, all mice received the dopa decarboxylase inhibitor NSD1015 (100 mg/kg intraperitoneally) and were then killed 30 min later. The striatum and n. accumbens were removed and frozen for analysis of l-dopa using high-performance liquid chromatography with electrochemical detection (HPLC-ECD) as previously described [7]. Significant differences were identified using two-way analysis of variance (ANOVA) followed by the Tukey-Kramer test; P < 0.05 was regarded as significant.

For microdialysis studies, male Sprague-Dawley rats (260-320 g; Charles River) were anaesthetized and their heads secured in a stereotaxic frame. A microdialysis probe was implanted into either the n. accumbens or striatum and secured in position (tip co-ordinates: n. accumbens, P +2.7, L +/− 1.6, V −7.4; striatum, P 0.0, L +/− 2.8, V −6.0; reference point Bregma according to [8]). Animals were allowed to recover for 24 h, after which probes were perfused with artificial cerebrospinal fluid (aCSF: NaCl 125 mM, KCl 2.5 mM, MgCl₂ 1.18 mM, CaCl₂ 1.26 mM, pH 7.4) at a flow rate of 2 µl/min, and 20-min dialysis samples were collected. After a 2-h stabilization period, three stable baseline control samples were taken. Agonist or vehicle was then administered subcutaneously and samples were collected for a further 3 h. For antagonist studies, three baseline control samples were taken followed by administration of antagonist or vehicle. Thirty minutes later agonist or vehicle was administered and samples collected for a further 3 h. The samples were assayed for dopamine by HPLC-ECD as previously described [7]. Data were analysed by two-way ANOVA with repeated measures followed by post-hoc testing using the Tukey-Kramer test; P < 0.05 was regarded as significant.

All compounds significantly (P < 0.05) decreased dopamine synthesis in the n. accumbens compared with vehicle controls with the following order of potency (ED₅₀, µg/kg s.c. in parentheses): (+)PHNO (0.55) > quinpirole (1.8) > apomorphine (21) = 7-OH-DPAT (23) = quinpirole (33). Dopamine synthesis in the striatum was decreased with a similar order of...
Table I

Comparison of effects of selective D3 and non-selective D2/D3 receptor agonists on dopamine synthesis in the rat n. accumbens and striatum

<table>
<thead>
<tr>
<th>Agonist</th>
<th>N. accumbens</th>
<th>Striatum</th>
<th>Striatum/n. accumbens ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-PHNO</td>
<td>0.55</td>
<td>1.4</td>
<td>2.6</td>
</tr>
<tr>
<td>Quinelorane</td>
<td>1.8</td>
<td>8.2</td>
<td>4.6</td>
</tr>
<tr>
<td>Apomorphine</td>
<td>21</td>
<td>64</td>
<td>3.0</td>
</tr>
<tr>
<td>7-OH-DPAT</td>
<td>23</td>
<td>75</td>
<td>3.3</td>
</tr>
<tr>
<td>(-)Quinpirole</td>
<td>33</td>
<td>48</td>
<td>1.5</td>
</tr>
</tbody>
</table>

potency (see Table 1), although quinpirole was slightly more potent than (+)-7-OH-DPAT and apomorphine. The similar ratio of striatum/n. accumbens for D3-selective ligands such as quinelorane as for non-selective ligands such as apomorphine suggests that there is no difference between the effects of these ligands in the two areas. Similar findings were observed by Svensson et al. [5] and Gainetdinov et al. [3]. Assuming that receptor reserve is similar in both regions (though this has yet to be demonstrated), this in turn suggests that dopamine synthesis may be regulated via D3 rather than D2 receptors. However, the lack of selective D3 receptor antagonists leaves this open to debate.

In microdialysis experiments (Figure 1), the D3 receptor agonist quinelorane and the D2/D3 receptor agonist quinpirole, both at a dose of 30 μg/kg subcutaneously, significantly (P < 0.05) decreased extracellular levels of dopamine in the n. accumbens and the striatum to a maximum of 40% of preinjection control values. Both compounds were without effect at lower doses of 1 and 10 μg/kg subcutaneously.

The D2 receptor antagonist GM 34333 [9] at a dose of 3 mg/kg subcutaneously, which had no significant effect per se, had no significant effect on the quinelorane-induced decrease in extracellular dopamine levels in the n. accumbens (Figure 2). As with the synthesis studies, the lack of differentiation between the effects of these compounds in the n. accumbens and striatum suggests that dopamine release is not regulated via D3 receptors. In addition, the doses of D3 and D2/D3 receptor agonists used to inhibit dopamine release (present data, [10,11]) are higher than those used to induce hypolocomotion [12], a behaviour suggested to be mediated by post-synaptic D3 receptors [5,13]. However, recent findings by Gainetdinov et al. [3], who measured interstitial levels of (+)-7-OH-DPAT by microdialysis after systemic administration of this compound, demonstrated that at doses of up to 0.12 μmol/kg intraperitoneally (+)-7-OH-DPAT is selective for the D3 receptor over the D2
Effects of the selective D_3 receptor antagonist GM 34333 on the quinelorane-induced inhibition of dopamine release in the rat nucleus accumbens

Mean±SEM of area under the curve n = 6 per group. *P<0.05 saline versus quinelorane.

By extrapolating these data to quinelorane (a compound demonstrated to be more selective for the D_3 receptor than (+)-7-OH-DPAT [6]), the doses used in the present study are also selective for the D_3 receptor. In support of quinelorane's effects being mediated via D_3 rather than D_2 receptors, the D_2 receptor antagonist GM 34333 was unable to block the quinelorane-induced decrease in dopamine release in the n. accumbens.

One possible explanation for the discrepancy between doses of D_3 ligands used in behavioural and neurochemical studies is that both the hypolocomotor effects and the decrease in dopamine release are mediated via D_3 receptors but that the hypolocomotor effects are mediated via post-synaptic and the release-modulating effects via presynaptic D_3 receptors. These different populations of receptors are differentially stimulated by low and high doses of D_3 receptor agonists respectively. This would explain the lack of effect of (+)-7-OH-DPAT on dopamine release when administered at a dose that correlated with the ED_{50} for hypolocomotor effects [5]. However, the lack of appropriate tools is a major drawback, and further studies using selective D_3 receptor antagonists are required to elucidate further the functional role of the D_3 receptor.


Received 11 August 1995