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<tr>
<td><strong>Authors(s)</strong></td>
<td>O'Connor, J. J.; Lowry, John P.</td>
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<tr>
<td><strong>Publication date</strong></td>
<td>2012-07</td>
</tr>
<tr>
<td><strong>Publication information</strong></td>
<td>European Journal of Pharmacology, 686 (1-3): 60-65</td>
</tr>
<tr>
<td><strong>Publisher</strong></td>
<td>Elsevier</td>
</tr>
<tr>
<td><strong>Item record/more information</strong></td>
<td><a href="http://hdl.handle.net/10197/4790">http://hdl.handle.net/10197/4790</a></td>
</tr>
<tr>
<td><strong>Publisher's statement</strong></td>
<td>This is the author's version of a work that was accepted for publication in European Journal of Pharmacology. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in European Journal of Pharmacology (Volume 686, Issues 1 3, 5 July 2012, Pages 60 65) DOI:10.1016/j.ejphar.2012.04.046Elsevier B.V.</td>
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<td><strong>Publisher's version (DOI)</strong></td>
<td>10.1016/j.ejphar.2012.04.046</td>
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</tbody>
</table>

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A comparison of the effects of the dopamine partial agonists aripiprazole and (-)-3-PPP with quinpirole on stimulated dopamine release in the rat striatum: studies using fast cyclic voltammetry in vitro

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Abstract
The effects of aripiprazole, (-)-(3-hydroxyphenyl)-N-n-propylpiperidine (-)-3-PPP and quinpirole on single and multiple pulse stimulated dopamine release were investigated using the technique of fast cyclic voltammetry (FCV) in isolated rat striatal slices. Aripiprazole and (-)-3-PPP had no significant effect on single pulse dopamine release at concentrations from 10nM to 10µM indicating low agonist activity. The compounds failed to potentiating 5 pulse stimulated release of dopamine although inhibitory effects were seen at 10 µM for aripiprazole. Both compounds were tested against the concentration-response curve for quinpirole’s inhibition of stimulated single pulse dopamine release. Aripiprazole and (-)-3-PPP shifted the concentration-response curve for quinpirole to the right. In each case this was a greater than a 100-fold shift for the 10 µM test compound. Whilst these results indicate that both compounds show little agonist activity on dopamine release and significant antagonism of the inhibitory effect of quinpirole on dopamine release, whether they are functionally selective dopamine D2 ligands remains controversial.

Key words: Aripiprazole, 3-PPP, quinpirole, voltammetry, dopamine release, partial antagonist, autoreceptor.

1. INTRODUCTION
Fast cyclic voltammetry (FCV) is a technique that can measure in real time the release of dopamine from rat striatal slices. It is therefore a useful tool to accurately assess the efficacy of dopamine D2 agonists and partial agonists on stimulated single and multiple pulse dopamine release (Palij et al., 1990; Bull & Sheehan, 1991; Trout and Kruk, 1992). Full agonism may be tested on single pulse stimulated release, as the endogenously released dopamine will have no effect on the dopamine D2 presynaptic autoreceptors. Full antagonism can be measured by either using a suitable multiple pulse protocol where endogenous dopamine D2 autoreceptors are activated, or assessing the ability of the compound in question to inhibit the effect of a full agonist such as quinpirole on dopamine release (Limberger et al., 1991).

Aripiprazole is an atypical antipsychotic and antidepressant used in the treatment of schizophrenia, bipolar disorder, and clinical depression. Aripiprazole's mechanism of action is different from those of other FDA-approved atypical antipsychotics (e.g., clozapine, olanzapine, quetiapine, ziprasidone, and risperidone). Rather than antagonizing the dopamine D2 receptor, aripiprazole acts as a dopamine D2 and 5-HT1A receptor partial agonist (Ki = 0.34 nM and 1.65 nM respectively, Lawler et al., 1999). It can significantly increase dopamine levels in the prefrontal cortex of rats but only at low concentrations (Zocchi et al., 2005).

(-)-3-PPP has also been shown to have some antipsychotic action but not sustained receptor desensitization. It has been used in schizophrenia possibly by attenuating dopamine function in two different ways, by stimulating the presynaptic receptors and blocking the postsynaptic receptors. It has previously been reported that in contrast to racemic 3-PPP, (+)-3-PPP can inhibit electrically evoked release of both [3H]dopamine and [14C]acetylcholine from superfused rat neostriatal slices (Mulder et al., 1985). In contrast (-)-3-PPP did not have inhibitory effects on dopamine release but antagonized those effects of (+)-3-PPP. Both enantiomers can reduce increases in striatal dopamine synthesis produced by γ-butyrolactone, although the (-)-enantiomer was only partially active (Clark et al., 1984). 3-PPP has also been shown not to protect against MPTP-induced dopaminergic neurotoxicity (Muralikrishnan et al., 2004). In contrast to the above compounds quinpirole has previously been shown by us and other groups to act as a full agonist inhibiting dopamine release in the rat striatum with high potency (for example see (Palij et al., 1990, O’Neill et al., 2009).

The determination of the functional intrinsic activity of partial agonist compounds at dopamine D2 receptors is a difficult task when carried out in brain
native tissues. The aim of the study was to quantify the potential partial agonist and antagonist activity of (-)-3-PPP and the dopamine D$_2$ partial agonist, aripiprazole, on pre-synaptic dopamine D$_2$ autoreceptors located in the terminals of dopamine neurons in the rat striatum using FCV. We have also compared the effect of these compounds with that of the full agonist quinpirole.

2. Materials and Methods

2.1. Electrochemical System

2.1.1. Generation of the signal

Fast cyclic voltammetry (FCV) is an electrochemical technique designed to enable detection of monoamine release in real time (see Stamford, 1990 for a comprehensive review). Its advantages are the speed of recording and the small size of the working electrode (typically 7 µm diameter by 50 µm length). A three electrode configuration is typically used in brain slice FCV, namely an auxiliary electrode, working electrode and reference electrode. The reference electrode is a silver/silver chloride electrode (A-M Systems, Inc, WA) whilst the auxiliary electrode is also a Ag/AgCl electrode but bridged from the bath in a plastic pipette tip filled with 1 M NaCl. Fast cyclic voltammetry (FCV) was carried out using a Millar Voltammeter (Dr. Julian Millar, Queen Mary & Westfield College, University of London, UK) connected to an FCV headstage (see Millar and Barnett, 1988 for a full circuit diagram of the apparatus). The FCV amplifier subjects the working electrode to a triphasic set of anodic and cathodic voltage sweeps in a period of 20 ms. The triphasic voltage waveform ramps from 0 to -1.0 V to +1.4 V to -1.0 V to 0 (20 ms) equating to a scan of 480 V/s. Throughout our experiments this waveform was applied to the potentiostat four times a second. Because the scan only lasts 20 ms, it can be repeated many times a second if required. The input triphasic ramp pattern for FCV in these experiments is shown in Fig. 1A. The FCV headstage circuit measures the working electrode current (generated by the drive voltage) and from this current the concentration of electrically oxidizable or 'electroactive' material at the tip of the working electrode can be computed.

2.1.2. Faradaic current

Following the triphasic voltage input into the working electrode a background current is monitored that is due to the complex impedance characteristics of the electrode/ electrolyte interface (Fig. 1B). When electroactive materials such as dopamine are present on the surface of the electrode extra current flow is generated through the electrode. This electron influx is known as the faradaic current (Fig. 1C) and includes both oxidation and reduction peaks, for dopamine, characteristically +600 mV and -200 mV respectively. To provide information on dopamine release dynamics, a sample and hold circuit was set to monitor current at +600 mV on each successive scan. The typical output from this display is shown in Fig. 1D in response to dopamine.

2.1.3. Carbon fiber electrode manufacture

Carbon fiber electrodes were home manufactured but see also Armstrong James & Millar (1979). A borosilicate capillary tube (1 mm i.d.) was filled with acetone and a single carbon fiber inserted (7 µm diameter). Upon drying the tube the capillary was pulled by an electrode-puller (P97, Sutter Instrument, Novato, USA) giving rise to two electrodes both with a glass seal around a single carbon fiber. The exposed length of the fiber was cut back mechanically under a microscope to give rise to an exposed length of approximately 50 µm. This enables the electrode to measure monoamines such as 5-hydroxtramine (O’Connor & Kruk, 1991; 1992) and dopamine (Kruk & O’Connor, 1995) in very specific nuclear regions of the brain.

2.2. Brain slice preparation

Male Wistar rats (178-283g) housed 4 to a cage were purchased by NUIM fortnightly from Harlan, UK and kept in the BioResource Unit at NUI Maynooth. All experimental procedures were approved by the Animal Research Ethics Committee of the Biomedical Facility at the National University of Ireland, Maynooth. Rats were killed by decapitation. The brain was quickly removed into ice-cold artificial cerebrospinal fluid. Blocks of tissue containing the caudate putamen and nucleus accumbens were prepared. 350 µm thick slices were sectioned using a Campden vibrotome. Brain slices were then transferred to a holding chamber containing artificial cerebrospinal fluid (see below) at room temperature (20-21°C) to equilibrate for 1 h. A single slice was then transferred to a recording chamber and perfused with oxygenated aCSF at 4 ml/min at 30-31°C for 40 min before electrical stimulation.

2.3. Measurement of endogenous dopamine release

Following 40 min equilibration, a bipolar tungsten-stimulating electrode with a tip separation of 200 µm (A-M Systems, Inc.) was placed in the dorsolateral caudate putamen (see Fig. 1). A carbon fibre electrode as described previously, was placed 100-200 µm from the stimulating electrode. Stimulated dopamine release (using Neurolog modules) was evoked using a square-wave pulse of 10 V amplitude and 100 µs duration delivered once every 2 min. A sample and hold output before during and after a stimulus is shown in Fig. 1D. Dopamine release under these conditions is tetrodotoxin-sensitive and Ca$^{2+}$-dependent (Palij et al., 1990). Sample and hold data was recorded onto a PC via a 4 channel LabChart. Recordings were taken in the dorsomedial striatum (Fig. 1E).

2
2.4. Carbon fiber electrode calibration

Electrodes were calibrated with increasing concentrations of freshly prepared dopamine in the range 0.05 µM to 1 µM, concentrations in the range of the endogenous dopamine released in the slices. The relationship of the dopamine concentration (µM) and the measured faradaic current (nA) was found to be linear in this range (see O’Neill and O’Connor, 2008).

2.5. Experimental Protocols

Single pulse dopamine was stimulated every 2 min during the course of the experiment. Every 30 min a multiple pulse stimulation protocol was carried out (5 pulses at 10 Hz). Dopamine D₂ antagonists have previously been shown to increase this signal (Limberger et al., 1991) but not by others (Trout and Kruk, 1992). Cumulative concentration response curves were carried out for each compound; 30 min baseline control, 30 min 100nM, 30 min 1 µM and 30 min 10 µM compound. In a final set of experiments 2 concentrations of quinpirole (30 min each) were added after pretreatment of the brain slices for 30 min with either 10 µM aripiprazole or -(3)-PPP. The maximum inhibition of dopamine release for each concentration of quinpirole was obtained from the average of the last two values during the 30 min perfusion.

2.6. Drugs and Materials

Artificial cerebrospinal fluid was prepared every day according to the following composition in mM: NaCl, 120; KCl, 2.5; MgSO₄, 2; CaCl₂, 2; NaH₂PO₄, 1.25 and D-glucose, 10 mM in H₂O. It was bubbled with 95% O₂/5% CO₂. Aripiprazole was a gift from Dr. Paolo Cavanni, GSK, Verona. (-)-3-PPP and quinpirole were obtained from Sigma (UK). Aripiprazole and (−)-3-PPP were dissolved in 100% dimethylsulphoxide to a concentration of 10⁻⁴M and stored at -20°C in 100µL volume containers. Dimethylsulphoxide final volume in aCSF was always <0.05%. Quinpirole was dissolved in artificial cerebrospinal fluid also to 10⁻⁵M. All compounds were used within 5 days of preparation. Stock solutions of dimethylsulphoxide were made to obtain final bath concentrations of dimethylsulphoxide lower than 0.005% in the superfusing artificial cerebrospinal fluid. Solvent controls were carried out with similar dimethylsulphoxide controls. Previous experiments
with 0.05% dimethylsulphoxide in the perfusing artificial cerebrospinal fluid did not affect single pulse dopamine release, rise time or decay time (Fig. 2; see also O’Neill & O’Connor, 2008).

2.7 Data analysis

All sample and hold data were analyzed and peak release measured. These values were exported into excel sheets. Single pulse evoked dopamine over flow was measured as the peak release in response to electrical stimulation. Rise time and half decay time of dopamine release were measured in some of the experiments. Rise time was measured from the beginning of baseline to peak amplitude and half decay time was measured from peak release to 50% half decay. Stimulated dopamine release was measured over 6 min (3 stimulations) prior to the first test drug application and the average of these 3 values were taken as 100%. All values prior and subsequent to these were represented as % control. Quinpirole EC50 curves were generated from the average % inhibition (last 2 values) at the end of each 30 min application of quinpirole. EC50 values were calculated using the software package Graph Pad Prism™. Data are presented as means±standard error of the mean (S.E.M.) of at least 4 independent experiments (different brain slices). Student’s t-test (paired and unpaired sampling where appropriate) was carried out at the individual time points indicated in the figures. P<0.05 was considered significant.

3. Results

3.1 Control experiments

Single pulse dopamine release was evoked every 2 min (10V; 0.1ms duration) and was stable for more than 2 hr. Typically evoked single pulse dopamine release ranged from 0.05 to 0.12µM dopamine when measured against the calibrated electrode. Rise and decay times ranged from 0.5 to 0.75 and 0.5 to 1 s respectively. Application of the solvent DMSO, used for the test compounds, at concentrations of 0.025 and 0.05%W/V had no effect on single pulse dopamine release (Fig. 2B). 5 pulses at 10 Hz, multiple pulse stimulation, was evoked every 30 min and was also stable for more than 2 hr (Fig. 2A and B). DMSO also had no significant effect on multiple pulse stimulation over this time period.

3.2 Effects of Aripiprazole, PPP and quinpirole on single and multiple pulse evoked dopamine release

Application of aripiprazole and (-)-3-PPP at concentrations ranging from 10^-8M to 10^-5M each for 30 min, did not have any effect on single pulse evoked dopamine release (Fig. 3A and B respectively). Aripiprazole at 10 µM but not 0.01 to 1.0 µM significantly inhibited multiple pulse stimulated dopamine release (5 pulses at 10 Hz; paired Student t-test; **P<0.01; Fig. 3A). Fig. 3C illustrates the inhibitory effect of quinpirole on single pulse dopamine release. Increasing concentrations of quinpirole (10 nM to 100 nM) were added to the brain slice chamber and complete inhibition of dopamine release occurred at 100 nM. An EC50 value of 32nM was calculated for quinpirole from the software package Graph Pad Prism™. This is in agreement with other publications using this technique (see O’Neill et al., 2009).

3.3 Effects of Aripiprazole and PPP on the inhibition of dopamine release by quinpirole

Two concentrations of quinpirole (1 and 10 µM) were applied for 30 min to brain slices, which, had been pretreated with 10 µM of either aripiprazole or (-)-3-PPP. Fig. 4A and B shows the averaged time course data showing the effect of 1.0 and 10 µM
quinirole on stimulated dopamine release in the presence of 10 µM aripiprazole (A) or (-)-3-PPP (B). EC$_{50}$ values for quinirole alone and in the presence of aripiprazole (10µM) were 38 nM and 7.4 µM respectively. This represents a 200 fold right-ward shift in the concentration response curve.

In the presence of quinirole on stimulated dopamine release at any of the concentrations tested.

C. Averaged time course data showing the effect of 10 and 100 nM quinirole on single pulse stimulated dopamine release. Quinirole had a potent inhibitory effect on dopamine release with 10 nM and 100 nM inhibiting release by 25 and 85% respectively as previously reported.

In the presence of (-)-3-PPP the EC$_{50}$ values were 38 nM and 5.6 µM (a 150 fold shift; Fig. 4C and D respectively).

**Figure 3.** The effect of aripiprazole, 3-PPP and quinirole on stimulated dopamine release in brain slices of the rat striatum.

- **A.** Averaged time course data showing the effect of 0.01, 0.1, 1.0 and 10 µM aripiprazole on stimulated dopamine release. The upper four points represent the 5-pulse protocol every 30 min. Aripiprazole did not have any effect on single pulse release at any of the concentrations. However 5 pulses at 10Hz was significantly inhibited at 10 µM only (paired Student t-test; *P<0.01; n=4).

- **B.** Averaged time course data showing the effect of 0.01, 0.1, 1.0 and 10 µM 3-PPP on stimulated dopamine release. The upper six points represent the 5-pulse protocol every 30 min. 3-PPP did not have any effect on single or multiple pulse release at any of the concentrations tested.

- **C.** Averaged time course data showing the effect of 10 and 100 nM quinirole on single pulse stimulated dopamine release. Quinirole had a potent inhibitory effect on dopamine release with 10 nM and 100 nM inhibiting release by 25 and 85% respectively as previously reported.

**Figure 4.** Effect of aripiprazole and 3-PPP in the presence of the dopamine D$_2$ receptor agonist quinirole on dopamine release.

- **A.** Averaged time course data showing the effect of 1 µM and 10 µM quinirole (indicated by black line bars) on stimulated dopamine release in the presence of 10 µM aripiprazole (present throughout the experiment).

- **B.** Averaged time course data showing the effect of 1 µM and 10 µM quinirole (indicated by black line bars) on stimulated dopamine release in the presence of 10 µM 3-PPP (present throughout the experiment). All data in A and B is presented as means±standard error of the mean (S.E.M.) of 4-5 independent experiments (different brain slices).

**C.** EC$_{50}$ curves for the effect of quinirole alone (0.01 and 0.1 µM; black squares, left) and quinirole in the presence of aripiprazole (10 µM, open circles; right) on single pulse dopamine release. IC$_{50}$ values were estimated using the software package Graph Pad Prism™. There is an approximate 200-fold shift to the right of the curve in the presence of 10 µM aripiprazole (see results section for values).

**D.** EC$_{50}$ curves for the effect of quinirole alone (0.01 and 0.1 µM; black squares, left) and quinirole in the presence of 3-PPP (10 µM, triangles; right) on single pulse dopamine release. There is an approximate 150-fold shift to the right of the curve in the presence of 10 µM aripiprazole. In both C and D results are expressed as percentage inhibition of dopamine release against log molar concentration of quinirole. All data is presented as the mean of 4 to 7 independent experiments (different brain slices).

**4. DISCUSSION**

Using the technique of fast cyclic voltammetry it was expected that the agonist activity of dopamine compounds would manifest in an inhibition of single pulse stimulated dopamine release. On the other hand if a multiple pulse protocol was used to stimulate dopamine release and thus activate dopamine D$_2$ autoreceptors, it might be expected that the antagonist activity of a test compound might increase stimulated dopamine release. Using FCV we also investigated if a partial antagonist applied at low concentrations might give rise to agonist activity alone whilst show antagonist activity at higher concentrations.

Surprisingly application of the standard dopamine D$_2$ partial agonist aripiprazole had no significant effect on single pulse release at concentrations from 10nM to 10 µM. Aripiprazole is known to be very active at dopamine D$_2$ receptors (pEC$_{50}$/pKi ~ 9.5/9.8) and a starting concentration of 10 nM is greater than 10 fold higher than its pEC$_{50}$ (Lawler et al., 1999). Therefore lower concentrations of this
partial agonist may be required to be tested (typically <1 nM) to see an agonist effect. Therefore from these data alone we cannot determine if aripiprazole is a typical dopamine D₂ agonist or a functionally selective dopamine D₂ ligand. Another reason higher concentrations did not give rise to an inhibition of dopamine release may be that other receptors can be consistently occupied at that concentration (10 to 100 nM). For example aripiprazole at this concentration may have small but significant effects on dopamine D₂, D₃, D₄, 5HT₁A/2A/2C/7, alpha₁B and H₁ receptors (Jordan et al., 2002). It is not known at this time if all of these receptors can directly or indirectly influence dopamine release. However in the region we are recording from it is unlikely that activation of all of these receptors plays a major role in the modulation of presynaptic dopamine release. Experiments using (-)-3-PPP (another known partial agonist) also did not detect inhibitory effects on single pulse dopamine release at 10 nM, 100 nM and 1 μM.

For both compounds the 5-pulse protocol, which was used to look at antagonist activity, did not show any significant increases in dopamine release in the presence of all four concentrations. This may be that the protocol is not suitable to observe these effects (see Limberger et al., 1991 and in contrast, Trout and Krak, 1992). Longer stimulation protocols such as 10 or 20 pulses at 10 to 100 Hz would have detrimental effects on the concurrent single pulse experiments and these would have to be separated out. Therefore this protocol may not be as useful as an assessment of antagonist activity. Because of this it was decided to test the antagonist effects of the compounds on the inhibition of dopamine release by quinpirole.

Both compounds when present at 10 μM caused an approximate 150 to 200-fold shift to the right of the quinpirole concentration-response curve which compares favorably with the actions of other full antagonists at dopamine D₂ receptors in previous work using this technique (Palij et al., 1990; Bull and Sheehan, 1991 in both the rat striatum and nucleus accumbens). Our results were similar to the antagonistic effects of sulpiride, metoclopramide and clozapine.

The fact that aripiprazole can modulate in vivo 5-HT and DA release in mPFC through the activation of 5-HT₁A receptors may be a concern (Bortolozzi et al., 2007). These effects of aripiprazole in the cortex were through partial agonist activity at dopamine D₂ autoreceptors and distinct from those of haloperidol. In a recent study a role for aripiprazole has been uncovered at dopamine D₃ autoreceptors also (Tadori et al., 2008). We did not investigate its actions at these receptors in this study. The new generation antipsychotics including aripiprazole all seem to preferentially increase dopamine output in the nucleus accumbens as compared to the striatum (Hertel, 2006). It would be interesting to look at the effects of these agents in the nucleus accumbens. Partial agonist properties of aripiprazole were not revealed in studies carried out by Koener et al., (2011) using guanosine 5'-O-(γ-[³²S]thiotriphosphate ([³²S]GTPγS) binding assays on striatal membranes from haloperidol-treated rats. This was also observed in behavioral assays. In fact aripiprazole behaved as an antagonist, efficiently inhibiting the functional response to dopamine.

In a micro-dialysis study by Oshibuchi et al., (2009), both aripiprazole and the full antagonist haloperidol equally suppressed increases in dopamine levels in fear conditioned rats. However only aripiprazole decreased tonic dopamine levels. This may indicate a differential effect of aripiprazole on tonic and phasic dopamine release. Whether aripiprazole is a typical dopamine D₂ partial agonist, or a functionally selective dopamine D₂ ligand, remains controversial (Urban et al., 2007). These authors have shown downstream signaling differences between aripiprazole and both quinpirole and (-)-3-PPP. Using G protein activation and prolactin release as a measure of dopamine D₂ receptor activation, Cosi et al., (2006) showed that aripiprazole acted as a partial agonist at dopamine D₂ receptors. It is also likely that sensitivity to aripiprazole is different between humans and rodents. In an interesting computer simulation report by Spiros et al., (2010), aripiprazole was tested at dopamine D₂ receptors and predicted to have greater dopamine D₂ receptor antagonist in the human than in the rodent.

Our results also indicate competitive antagonism for the effects of (-)-3-PPP on the inhibition of dopamine release by quinpirole. Early reports on the activity of (-)-3-PPP at dopamine D₂ receptors are also equivocal. Plantje et al. (1983; 1984), similar to our data, were able to demonstrate an antagonist effect of (-)-3-PPP in the rat striatum investigating the inhibition of K⁺-induced [³H]acetylcholine release caused by the dopamine D₂ receptor agonist LY 141865. In the same year Markstein and Lahaye (1983) observed contrasting results where (-)-3-PPP did not inhibit electrically-evoked tritium overflow from rat striatal slices pre-incubated with [³H]dopamine. Arilla and Langer (1984) investigated the action of both enantiomers of 3-PPP on spontaneous and electrically evoked HW dopamine release in the striatum of rabbits. (-)-3-PPP increased spontaneous dopamine overflow but inhibited electrically evoked dopamine release. In contrast to (+)-3-PPP, (-)-3-PPP increased electrically evoked dopamine release with no effect on spontaneous overflow. Furthermore similar to the findings presented here, (-)-3-PPP antagonized the inhibitory effect of apomorphine, d-amphetamine and (+)-3-PPP on evoked dopamine release. Finally Stamford et al., (1991) working in vivo and using a longer stimulation protocol observed increases in dopamine release in the striatum and nucleus accumbens in the presence of (-)-3-PPP. The (+) enantiomer presumable acting as a full agonist caused a decrease in release in the nucleus accumbens and little effect in the striatum.
5. Conclusion

To date there has been equivocal data on the actions of both aripiprazole and (-)-3-PPP as partial agonists at dopamine D₂ presynaptic autoreceptors. Our results indicate that whilst both compounds show little agonist activity on single pulse dopamine release at low and high concentrations, they have significant antagonistic properties at dopamine D₂ auto-receptors.

Acknowledgements

We would like to thank P. Cavanni and C. Large for discussions on the work and M. Dalton for technical assistance.

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