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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 potentiate 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
release characteristics, a sample and hold circuit was set respectively.

oxidation and reduction peaks, for dopamine faradaic current (Fig 1B) and includes both redox 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. 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-hydroxytramine (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. 1A). A carbon fibre electrode as described previously, was placed 200-200 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 Ca²⁺-dependent (Palić et al., 1990). Sample and hold data was recorded onto a PC via a 4 channel MacLab. Recordings were taken in the dorsomedial striatum (Fig. 1E).
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 pre-treatment 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 10-3 M 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 10-3 M. 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

Figure 1. Waveforms used in Fast Cyclic Voltammetry (FCV)

A. A triphasic voltage ramp is passed down the carbon fibre electrode four times per second (4 Hz). The ramp sweeps from 0 V (relative to silver/silver chloride reference electrode) to -1.0 V to +1.4 V to -1.0 V and back to 0 V. This sweep lasts 20 ms.

B. The resultant current measured by the carbon fibre electrode in called the charging current. Superimposed on the charging current is the current obtained when the electrode is placed in a ringer solution containing 1 μM dopamine.

C. If the charging current in B in the absence of dopamine is subtracted from that current in the presence of dopamine a trace typical of C is the result (subtractogram). This is known as the faradaic current and is the result of the oxidation and reduction of dopamine on the surface of the carbon fibre electrode. Dopamine oxidizes at approximately +610 mV and is reduced at approximately -200 mV.

D. The trace illustrated in D is the result of a sample and hold device measuring at +610 mV during the electrical stimulation of the striatum. The arrow indicates the time of stimulation of striatum (0.1 ms pulse width, 10 V). Post calibration of the CFMe indicated that approximately 0.1μM dopamine is evoked by a single electrical stimulation in the dorsolateral striatum. Peak rise time is approximately 0.5 s and half decay time approximately 1.0 s.

E. Placement of electrodes

Schematic diagram illustrating the placement of the carbon fibre microelectrode (recording electrode) and the bipolar stimulating electrodes (tip separation 200 μm) in the dorsolateral striatum. The carbon fibre electrode was placed 100 to 200 μm from the bipolar stimulating electrodes most commonly in the region illustrated.
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 values were calculated 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). Students 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 10⁻⁷ 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
that the antagonist activity of a test compound dopamine single compounds would manifest in an inhibition of was expected that. Using the technique of fast cyclic voltammetry it previously reported.

Figure 3. The effect of aripiprazole, 3-PPP and quinpirole on stimulated dopamine release in the presence of 10 µM aripiprazole (A) or (-)-3-PPP (B). EC50 values for quinpirole 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 (-)-3-PPP the EC50 values were 38 nM and 5.6 µM (a 150 fold shift; Fig. 4C and D respectively).

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 D2 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 D2 partial agonist aripiprazole had no significant effect on single pulse release at concentrations from 10 nM to 10 µM. Aripiprazole is known to be very active at dopamine D2 receptors (pEC50/pKi ~ 9.5/9.8) and a starting concentration of 10 nM is greater than 10 fold higher than its pEC50 (Lawler et al., 1999). Therefore lower concentrations of this
partial agonist may be required to be tested (typically <1nM) 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, alpha1A/1B 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 (Pålji 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-thiotriphosphate ([γ-32P]GTPyS) 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. Arbilla and Langer (1984) investigated the action of both enantiomers of 3-PPP on spontaneous and electrically evoked 3H 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.

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