Therapeutic doses of buspirone block D3 receptors in the living primate brain

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Abstract

Dopamine D3 receptor (D3R) antagonists may be effective medications for multiple substance use disorders (SUDs). However, no selective D3R antagonists are currently available for clinical testing. Buspirone, originally characterized as a 5-HT1A partial agonist and used as an anxiolytic, also binds to D3R and D4R with high affinity, with lower affinity to D2R, and interferes with cocaine reward. Here we used PET with [11C]PHNO (D3R-preferring radioligand), [11C]raclopride (D2R/D3R radioligand) and [11C]NNC-112 (D1R radioligand) to measure occupancy of oral and parenteral buspirone in the primate brain. Intramuscular buspirone (0.19 and 0.5 mg/kg) blocked both [11C]PHNO and [11C]raclopride binding to striatum, exhibiting high occupancy (50–85%) at 15 min and rapid wash-out over 2–6 h. In contrast, oral buspirone (3 mg/kg) significantly blocked [11C]PHNO binding in D3-rich regions (globus pallidum and midbrain) at 3 h, but had minimal effects on [11C]raclopride binding (28–37% at 1 h and 10% at 3 h). Buspirone did not block [11C]NNC-112. Our findings provide evidence that i.m. buspirone blocks D3R and D2R, whereas oral buspirone is more selective towards D3R blockade in vivo, consistent with extensive first pass metabolism and supporting the hypothesis that its metabolites (5- and 6′-hydroxybuspirone) merit evaluation for treating SUDs. They also indicate that for oral buspirone to achieve greater than 80% sustained D3R occupancy, as might be needed to treat addiction, higher doses (at least three-fold) than those used to treat anxiety (maximal 60 mg) will be required. Nonetheless, based on previous clinical studies, these doses would be safe and well tolerated.

Key words: Addiction, buspirone, dopamine receptors, 6′-hydroxybuspirone.

Introduction

There is an urgent need for better addiction treatments. The economic costs as a result of substance use disorders (SUD) (e.g. health care, lost productivity, criminal justice) are estimated at more than half a trillion dollars annually in the United States (Volkow and Li, 2005). Despite a devastating impact on public health, there are very few medications for the treatment of SUD, and these are restricted to alcohol, nicotine and opiate use disorders.

Insights into the neurobiological bases of addiction have highlighted the need for medications, which are able to either, reverse or compensate for addiction related changes in brain function (Kalivas and Volkow, 2011). Among these changes, mitigating the disruption of dopamine modulated striato-cortical circuits produced by chronic exposure to addictive drugs is an attractive target for medication development. This circuitry plays a critical role in the ability to control strong urges, and its disruption is implicated in the inability of an addicted person to stop using drugs, resulting in compulsive administration despite awareness of its potentially catastrophic consequences.

There are now converging lines of evidence that signalling mediated by the dopamine D3 receptor (D3R) is critical to the addictive process. For example, an up-regulation of D3R has been reported in post-mortem brains of individuals addicted to cocaine (Staley and Mash, 1996). Moreover, a recent positron emission tomography (PET) imaging study using the D3-prefering radioligand [11C]PHNO ([11C](+)-4-propyl-9-hydroxy-5-naphthoxazine) demonstrated an up-regulation of D3R in the midbrain and globus pallidum of methamphetamine abusers (Boileau et al., 2012). Pre-clinical studies have also demonstrated increases in D3R following chronic exposure to psychostimulant drugs (Caine and Koob, 1993; Neisewander et al., 2004; Heidbreder and Newman, 2010). These data, taken together with the
highly concentrated localization of D3R in regions of the brain reward circuitry (midbrain, ventral striatum, pallidum) (Sokoloff et al., 1990; Murray et al., 1994) has generated interest in investigating the potential of D3R as a molecular target for addiction treatments (Heidbreder and Newman, 2010). Indeed, in pre-clinical models D3R antagonists interfere with both self-administration (especially when the work demand for the reinforcer is increased) and relapse (reinstatement) against multiple drugs of abuse (Le Foll et al., 2005; Heidbreder and Newman, 2010).

While multiple D3R antagonists are available for pre-clinical studies (Heidbreder et al., 2005; Newman et al., 2012), only one report of a compound (GSK 598809) with the appropriate safety profile for use in humans has been published (Mugnaini et al., 2013). Consistent with the pre-clinical profile of D3R antagonists, GSK 598809 produced a transient reduction in craving for cigarettes in smokers after overnight abstinence. This effect was produced when occupancy of D3R assessed with PET and [11C]PHNO was estimated to be 72–89%. Further development of this compound was, however, halted by the sponsor (Newman et al., 2012).

Buspirone (Buspar®), an anxiolytic initially described as a selective 5-HT1A partial agonist (Wong et al., 2007; Loane and Politis, 2012), has been shown by in vitro studies to also bind to D3 receptors (D3R, Ki = 98 nM) and D4 receptors (D4R, Ki = 29.2 nM) with affinities similar to those reported at 5-HT1A receptors (Ki = 4–78 nM), and behaves as an antagonist at these dopamine receptors (Bergman et al., 2013). Buspirone also binds to D2 receptors (D2R, Ki = 484 nM), albeit with a much lower affinity compared to 5-HT1A, D3R, and D4R. Consistent with this neurochemical profile, buspirone (0.1–0.32 mg/kg, i.m.) inhibited self-administration of cocaine at all unit doses in rhesus monkeys (Bergman et al., 2013; Mello et al., 2013) and interfered with relapse to stimulant abstinence (Newman et al., 2012).

In this preliminary study, we tested the hypothesis that buspirone at doses within the range used clinically induces significant occupation of D3R in the living non-human primate brain, which would support its use in clinical trials for the treatment of addiction and provide information regarding dosing. To test this hypothesis, we used PET in conjunction with the D3R-preferring radiotracer, [11C]PHNO (Kd = 0.07 nM for D3R; 148-fold selectivity for D3R over D2R) (Wilson et al., 2005b; Parker et al., 2006), to measure D3R occupancy by buspirone and compared it with the occupancy derived with [11C]raclopride, which binds to D2R (1.5–1.6 nM) and D3R (1.2–2.1 nM) almost equally (Malmberg et al., 1994; Seeman, 2001). In addition, the distinct localization of D2R and D3R in the basal ganglia and the midbrain provides another means of distinguishing the binding of [11C]raclopride and [11C]PHNO to D2R vs. D3R. Thus, binding of [11C]PHNO in dorsal striatum, which has a much higher expression of D2R relative to D3R, is likely to reflect binding to both receptors whereas binding in the midbrain and pallidum is likely to predominantly reflect occupation of D3R (Tziortzi et al., 2011). We also measured in vivo binding of buspirone on D1 receptors (D1R) using a D1R selective radioligand, [11C]NNC-112 (Halldin et al., 1998). D1R concentration is high in mesolimbic areas and is implicated in cocaine reward and addiction (Campbell et al., 1999; Edwards et al., 2007; Graham et al., 2007).

We hypothesized that buspirone would bind to D3R in basal ganglia, thus interfering with the binding of [11C]PHNO, and that the duration of the binding would be longer lasting in D3R-rich regions (midbrain and pallidum) compared to D2R-enriched regions (dorsal striatum). We predicted that buspirone’s inhibition of specific binding of [11C]raclopride (reflecting combination of D2R and D3R) would be short-lived compared to that of [11C]PHNO (predominantly reflecting D3R). The effect of buspirone and its metabolites would be predicted to persist in D3-enriched areas, because their higher affinities for D3 relative to D2 receptors (Bergman et al., 2013) would result in a slower rate of dissociation. Our initial experimental design aimed to assess the effects of parenteral buspirone in blocking D3R; but after observing significant blockade of D2R (assessed with [11C]raclopride) we decided to also measure the effects of orally administered buspirone. This was relevant since oral buspirone is subject to extensive first pass metabolism (Gammans et al., 1986; Jajoo et al., 1989) and its metabolites bind with higher affinity for D3R relative to D2R (Bergman et al., 2013). Indeed the affinity of 6-hydroxybuspirone, which reaches plasma concentrations ~40-fold higher than buspirone after oral administration of the parent drug (Dockens et al., 2006), for D3R (795 nM) is higher relative than that of D2R (5390 nM) (Bergman et al., 2013). While plasma concentrations of 5-hydroxybuspirone, approximate those of the parent compound following oral administration (Gammans et al., 1986); it could also contribute based on its higher affinity for D3R (261 nM) relative to D2R (4010 nM) (Bergman et al., 2013).

**Materials and method**

**Ethics statement**

All baboons (Papio Anubis) were maintained at Brookhaven National Laboratory (BNL) Animal Facility in accordance with the United States Department of Agriculture (USDA) and New York State Department of Health animal welfare regulations. BNL is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, and maintains an assurance with the Office of Laboratory Animal Welfare (OLAW). All work conducted on the animals used in this study was performed under protocols.
approved by the BNL Institutional Animal Care and Use Committee (IACUC). Briefly, the baboons used in this study were singly housed in stainless steel caging that provided enrichment and interaction for each animal. The baboon’s daily diet consisted of a commercial primate diet (Fiber Plus Primate, Purina Mills, USA) along with supplemental fresh fruits and vegetables and other food enrichment. Fresh drinking water was provided ad libitum via an automatic watering system. All husbandries, environmental enrichment, veterinary, and other such procedures were performed in compliance with the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act. Animal health was monitored daily by certified animal care staff and veterinary personnel, available 24/7. Baboons showing signs of disease or distress that could not be alleviated using standard analgesics and/or chemotherapy were humanely euthanized using an overdose of barbiturates according to the guidelines of the American Veterinary Medical Association. It is noteworthy, however, that none of the animals became ill and/or met the criteria for the IACUC approved end-point policy during this study period, therefore, none were euthanized.

Subjects

Seven female baboons (13.5–21 kg) were studied in 51 PET scans (90 min scan) in which 3 radiotracers were administered with or without buspirone pre-treatment. Table 1 summarizes these studies. One baseline scan for each radiotracer and for each animal was performed and used for the occupancy calculation that resulted from buspirone administration. For [11C]raclopride and [11C]NNC-112 studies, a maximum of three sequential PET scans were performed 2 h apart from each other prior to and after buspirone pre-treatment. For [11C]PHNO, only one scan was performed per day and the time interval between scans was at least 3 wk to minimize ‘mass carry-over effect’ between scans in the same subject (Girgis et al., 2011; Gallezot et al., 2012; Searle et al., 2013). In order to minimize ketamine-induced endogenous dopamine receptor occupancy (Smith et al., 1998), ketamine was administrated at least 3.5 h before [11C]PHNO.

Drugs

Buspirone hydrochloride (purity>99.0%) was purchased from Sigma Aldrich and was used without further purification. Buspirone was administered orally, intramuscularly or intravenously. Estimated as free-base, buspirone was weighed and dissolved into 2.0 ml of sterile saline. This solution was then passed through a sterilizing filter (25 mm housing, Pall Co., USA) into a sterile, pyrogen-free vial. Oral doses were dissolved in saline (10 ml). 6′-hydroxybuspirone (hydrogen chloride salt form) was obtained from Dr Rik Kline (Chemistry and Pharmaceutics Branch, National Institute on Drug Abuse). 6′-hydroxybuspirone (1 mg/kg) was dissolved in saline (3 ml) and passed through a filter (25 mm housing, Pall Co., USA) into a sterile vial.

Radiotracer synthesis

[11C]PHNO (Wilson et al., 2005a), [11C]raclopride (Ehrin et al., 1987), and [11C]NNC-112 (Halldin et al., 1998) were prepared as previously described. [11C]Raclopride and [11C]NNC-112 precursors were purchased from ABX (Radeberg, Germany). [11C]PHNO precursor was provided by the Organic and Medicinal Chemistry Discovery and Analytical Sciences Program, RTI International (USA). After purification of the radiotracers by high performance chromatography, the HPLC solvent was evaporated under the reduced pressure. All radiotracers were formulated using saline (8 ml) and filtered through a sterile filter (Acrodisc® 13 mm with 0.2 μm HT Tuffryn® Membrane, Pall Co., USA) to give a sterile, pyrogen-free injection solution. The radiochemical purity was >99%.

PET imaging

Dynamic PET scans were performed on a Siemens HR+ high-resolution scanner (4.5×4.5×4.8 mm at the centre of the field of view, 63 planes) in 3-dimensional acquisition mode. A transmission scan was obtained with a 68Ge rotating rod source prior to radiotracer injection to correct for attenuation of the emission scans. The baboons were anesthetized with a dose of ketamine (10 mg/kg); intubated and ventilated with a mixture of isoflurane (1–4%, Forane; Baxter Healthcare Corp.), nitrous oxide (1500 ml/min), and oxygen (800 ml/min); and then catheterized for radiotracer injection as previously described (Kim et al., 2009). For the [11C]PHNO scans, baboons

<table>
<thead>
<tr>
<th>Radiotracer</th>
<th>Buspirone (mg/kg)</th>
<th>Pre-treatment (h)</th>
<th>Route</th>
<th># of scans*</th>
</tr>
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<tr>
<td>[11C]Raclopride Baseline</td>
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<td>4</td>
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<td>0.5</td>
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<td>0.50</td>
<td>2, 4, 6</td>
<td>i.m.</td>
<td>3×3</td>
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<td>3.0</td>
<td>1, 3, 5</td>
<td>p.o.</td>
<td>3×3</td>
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<tr>
<td>[11C]PHNO Baseline</td>
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<td>2</td>
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<tr>
<td>[11C]NNC-112 Baseline</td>
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* At least one baseline scan was obtained for each baboon.
were injected with 1.9–2.5 mCi [specific activity (SA) at the time of injection, 0.4–1.1 Ci/μmol]. The injected mass of $^{11}$C]PHNO for the baseline and the buspirone pre-treatment studies were 0.07±0.01 μg/kg and 0.05±0.02 μg/kg, respectively. The SA of $^{11}$C]raclopride and $^{11}$C]NNC-112 ranged between 1–2 Ci/μmol at the time of injection. Scanning was performed for 90 min with the following time frames (1×10 s, 12×5 s, 1×20 s, 1×30 s, 8×60 s, 4×300 s and 8×450 s). To assess buspirone occupancy, buspirone was administered i.m. (0.19, 0.5 mg/kg), orally (p.o. 3 mg/kg) and, for one study, i.v. (0.19 mg/kg) at different time points before the radiotracer injection (Table 1). We obtained a total of 29 scans with $^{11}$C]raclopride, 10 scans with $^{11}$C]PHNO and 12 scans with $^{11}$C]NNC-112. The oral doses of buspirone were administered through a nasogastric tube, which was flushed with saline (10 ml) as described elsewhere (Volkow et al., 1998). Additionally, we did one study to assess the effects of 6'-hydroxybuspirone (1 mg/kg), which was administered i.m. 3 h prior to $^{11}$C]PHNO injection (injected mass, 0.9 μg).

**Image analysis**

PMOD (PMOD Technologies Ltd, Switzerland) was used for image analysis. All reconstructed image data were converted to the ANALYZE format and co-registered with H2 15O template images as described previously (Kim et al., 2007). Regions of interest (ROIs) were placed over the caudate (CD), putamen (PU), globus pallidum (GP), midbrain [centre in substantia nigra, (SN)] and the cerebellum (CB) in the summed baseline images and adjusted manually from an MR template (Black et al., 2001). These ROIs were projected onto the co-registered dynamic images to obtain time-activity curves (TACs). Averaged TACs for the standard uptake values (SUV) of each radiotracer were computed to assess differences between baseline and buspirone. The simplified reference tissue model (SRTM) (Lammersma and Hume, 1996; Shotbolt et al., 2012) was applied into each ROI to obtain the non-displaceable binding potential (BPnd) using the cerebellum as a reference tissue for all three radiotracers. Differences in BPnd between control and buspirone pre-treatment scans were used to estimate drug occupancies as follows:

\[
\text{Occupancy}(\%) = 100 \times \frac{\text{BP_{baseline}}}{\text{BP_{baseline} + BP_{buspirone}}} - \frac{\text{BP_{buspirone}}}{\text{BP_{buspirone} + BP_{baseline}}} \]

The small sample sizes did not allow us to evaluate differences across route, dose and time periods. For statistical comparisons between the baseline and the buspirone scans we used repeated ANOVA when we had more than two time-points (i.m. buspirone 0.25 and 2.25 h) or doses (i.m. 0.19 and 0.5 mg/kg) followed by post-hoc $t$ tests and we used paired $t$ tests for the measures where only one time-point or one dose; this was the case for oral buspirone’s effects on $^{11}$C]PHNO binding.

**Results**

**Effects of buspirone (IM and PO) on radiotracer binding**

The TACs of $^{11}$C]raclopride (Fig. 1) and $^{11}$C]PHNO (Fig. 2) were significantly decreased by parenteral buspirone administration in basal ganglia but not in cerebellum.

Parenteral buspirone significantly reduced $^{11}$C]raclopride’s TACs in putamen for the 0.19 mg/kg ($t=1.8, p=0.05$) and the 0.5 mg/kg ($t=2.6, p=0.01$) doses, but not for the oral dose of buspirone ($t=0.24$, NS).

Parenteral buspirone significantly reduced TACs for $^{11}$C]PHNO in putamen and globus pallidum for the 0.19 mg/kg ($t=1.8, p=0.05$ and $t=3, p=0.005$, respectively) and the 0.5 mg/kg doses ($t=2.6, p=0.01$ and $t=4.45, p=0.0002$), while the effects in cerebellum were not significant ($t<1.2, p>0.13$). Similarly, oral buspirone (3 mg/kg) significantly decreased TACs for $^{11}$C]PHNO in putamen ($t=1.8, p=0.05$) and globus pallidum ($t=3, p=0.005$) and showed a trend in cerebellum ($t=1.6, p=0.06$) (Fig. 2).

The trend of an effect in cerebellum is consistent with the low density of D3R in cerebellum detected by $^{11}$C]PHNO.

The BPnd images of $^{11}$C]raclopride and $^{11}$C]PHNO are shown in Fig. 3 and reveal that i.m. buspirone decreased $^{11}$C]raclopride’s and $^{11}$C]PHNO’s specific binding whereas oral buspirone decreased $^{11}$C]PHNO but had minimal effects in $^{11}$C]raclopride’s specific binding.

Statistical analysis to assess the effects of buspirone on $\text{BP}_{\text{ND}}$ for $^{11}$C]raclopride (individual data shown in Supplemental Table 1) revealed that for the 0.19 mg/kg i.m. dose ($n=3, 3$ repeated measures) there was a significant main effect in caudate ($F=54, p=0.001$) and putamen.
Post-hoc t-tests showed significant reductions in caudate after 0.25 h ($t=9.5$, $p=0.01$) and 2.25 h ($t=5.6$, $p=0.008$) and in putamen after 0.25 h ($t=8.9$, $p=0.01$) and a trend at 2.25 h ($t=3.2$, $p=0.08$). For the 0.5 mg/kg IM dose ($n=3$, 4 repeated measures) there was a significant main effect in caudate ($F=11.5$, $p=0.007$) and putamen ($F=10.6$, $p=0.008$). Post-hoc t-tests showed significant reductions in caudate after 2 h ($t=4.5$, $p=0.04$), 4 h ($t=6.2$, $p=0.03$) and 6 h ($t=5.6$, $p=0.008$) and in putamen after 2 h ($t=4.9$, $p=0.04$), 4 h ($t=11$, $p=0.008$) and a trend at 6 h ($t=3.5$, $p=0.07$). For oral buspirone ($n=4$, 4 repeated measures) the main effect was significant in caudate ($F=7.6$, $p=0.008$) and putamen ($F=5.8$, $p=0.02$) and the post-hoc t-tests showed that decreases were significant only at 5 h in caudate ($t=3.2$, $p=0.05$) and putamen ($t=4.5$, $p=0.02$).

Statistical analysis (paired t-tests, $n=2$) to assess the effects of buspirone on BP$_{ND}$ for $[^{11}C]$PHNO (Supplementary Table 2) showed that for i.m. only the comparisons between baseline and the 0.5 mg/kg i.m. dose in midbrain (where the substantia nigra is located) was significant ($t=12$, $p=0.05$) whereas for oral buspirone the decreases were significant in putamen ($t=37$, $p=0.02$) and midbrain ($t=59$, $p=0.01$) and showed a trend in GP ($t=9.2$, $p=0.07$).

Neither i.m. or oral buspirone changed TACs nor BP$_{ND}$ of $[^{11}C]$NNC-112 in any of the brain regions measured (data not shown).

**Receptor occupancies with parenteral buspirone**

Buspirone reduced $[^{11}C]$raclopride BP$_{ND}$ at 0.25 h after pre-treatment, corresponding to a receptor occupancy in putamen (PU) of 77% after i.v. (0.5 mg/kg, $n=1$) and 77±10% after i.m. (0.19 mg/kg, $n=3$) and at 2.25 h the occupancy after i.m. (0.19 mg/kg) had decreased to 40±19% (Fig. 4). For the higher i.m. buspirone dose (0.5 mg/kg) the occupancy in putamen at 2 h was 54±15%, at 4 h it was 46±16% and at 6 h was 35±19%.
Results in caudate are similar to those in putamen (Fig. 4).

Buspirone (0.19 mg/kg, i.m.) reduced $[11C]$PHNO $B_{\text{ND}}$ at 0.25 h after pre-treatment, corresponding to an occupancy of 63% in PU and 61% in globus pallidum (GP) and by 2 h the occupancy had decreased to 20±6% ($n=2$) (Fig. 5). For the higher buspirone dose (0.5 mg/kg i.m. $n=2$) the occupancy at 2.25 h was 42±15%. Results in caudate are similar to those in putamen (Fig. 5 and Supplementary Table 2).

Receptor occupancies with oral buspirone

The low dose of oral buspirone (1 mg/kg, $n=1$, data not shown) produced minimal decreases in $[11C]$PHNO $B_{\text{ND}}$ with estimated occupancies at 1 h post-administration in caudate of 14%, in PU of 9% and in GP of 20%. The higher dose (3 mg/kg, $n=3$) resulted in significant blockade of $[11C]$PHNO $B_{\text{ND}}$ when measured 3 h after its administration corresponding to occupancies of 44±19% in PU, 55±12% in GP and 74±13% in midbrain (location of SN) (Fig. 5).

The higher dose of oral buspirone (3 mg/kg, $n=4$) elicited minimal blockade of $[11C]$raclopride’s specific binding 1 h post-administration (caudate: 29±18%; putamen: 37±32%) that had mostly cleared after 2 h post-administration (caudate 10±6%; putamen 10±10%) (Fig. 4). Since the high dose had minimal effects on $[11C]$raclopride’s $B_{\text{ND}}$, the effects of the low buspirone dose (1 mg/kg) were not examined.

Comparisons of $D_3$R occupancy between i.m. and oral buspirone showed it was significantly higher after i.m. (2 h measures) than after oral buspirone (3 h measures; $n=3$) in caudate (55±12% vs. 10.5±8%; $t=4.2$, $p=0.05$) and in putamen (64±16% vs. 11±12%; $t=4.1$, $p=0.05$).

Receptor occupancy with i.m. 6'-hydroxybuspirone

6'-Hydroxybuspirone (1 mg/kg) administered i.m. 3 h prior to $[11C]$PHNO injection blocked binding in midbrain (occupancy 89%) but showed low levels of occupancy in GP (20%).

Discussion

Here, we document that buspirone (oral and parenteral) significantly blocks $D_3$R in the living baboon brain at doses that are clinically relevant. We also demonstrate higher selectivity of oral buspirone for $D_3$R relative to $D_2$R when compared to i.m. buspirone. Consistent with the relatively low affinity of buspirone for $D_3$R in vitro (Bergman et al., 2013), we found no blockade of $D_3$R by buspirone.

Specifically, our preliminary data show that parenteral buspirone at doses that interfere with cocaine reward in rodents and in non-human primates (Bergman et al., 2013; Mello et al., 2013), rapidly blocked $D_3$R in the living non-human primate brain as evidenced by a reduction in binding of the $D_3$R-prefering radiotracer $[11C]$PHNO. This in vivo experiment extends previous in vitro studies.
demonstrating that buspirone exhibits high affinity for recombinant D3R (Bergman et al., 2013). The fast occupancy of D3R by oral and i.m. buspirone is consistent with pharmacokinetics studies that reported peak plasma buspirone concentrations after oral administration within 1 h in humans (Gammans et al., 1986) and non-human primates (Marathe et al., 1999). We were unable to find a pharmacokinetic analysis for i.m. buspirone, but since pharmacologically relevant concentrations have been reported within 15 min of i.m. buspirone (Bergman et al., 2013) we predict that peak concentrations after i.m. buspirone will be reached sooner than after oral buspirone. Future studies evaluating occupancies at different time-points are necessary to estimate the pharmacokinetics for the occupancy of D3R by buspirone in brain.

We also showed that i.m. buspirone produces a significant blockade of [11C]raclopride binding. In contrast, oral buspirone had a minimal effect in [11C]raclopride binding, showing a higher selectivity of D3R blockade over D2R blockade than i.m. administration. Indeed, despite the small sample size (n=3) the difference on D2R occupancies in dorsal striatum between oral and i.m. was significant. Similarly, despite the small sample sizes (n=2) we showed that oral buspirone significantly reduced [11C]PHNO binding in midbrain and showed a lower trend in pallidum (areas rich in D3R). Our ability to show significant differences even with the small sample sizes indicates that these are large effects.

The higher selectivity of oral buspirone for D3R relative to i.m. buspirone is consistent with the extensive first pass metabolism of orally administrated buspirone, which limits its bioavailability of the parent compound (only 4% in humans) (Mayol et al., 1985), and results in two active metabolites (5- and 6′-hydroxybuspirone) that possess a higher affinity for D3R (5-hydroxybuspirone, Ki=261 nM; 6′-hydroxybuspirone, Ki=795 nM) relative to D2R (5-hydroxybuspirone, Ki=4010 nM; 6′-hydroxybuspirone, Ki=5390 nM) in vitro (Bergman et al., 2013). Prior PET studies had reported differences between the parent compound and its metabolite for clomipramine and desclomipramine (Takano et al., 2011) and for quetiapine and norquetiapine (Nyberg et al., 2013).

It is important to note that buspirone and the 5′- and 6′-hydroxy metabolites also possess high affinity and antagonistic efficacy for D3R in vitro (5-hydroxybuspirone, Ki=107 nM; 6′-hydroxybuspirone, Ki=40 nM) (Bergman et al., 2013). Since there is in vivo evidence that D3R antagonists are effective (Feldpausch et al., 1998; Yan et al., 2012) in animal models of SUD, this highlights a potential role of D3R antagonism in the anti-addictive properties of buspirone. Moreover, given that the affinity of 6′-hydroxybuspirone, a metabolite with a total plasma exposure ~40-fold higher than buspirone following oral administration (Dockens et al., 2006) approximates that of the parent drug at D3R (Bergman et al., 2013), it is likely that D3R occupancy would be similar or higher than at D2R.

Pharmacokinetic differences in drug delivery into the brain are also likely to contribute to higher D2R binding for i.m. than oral administration since IM buspirone would result in higher and faster brain levels of the parent compound than with oral buspirone, which is extensively metabolized. However, for D3R occupancy, the higher affinity of buspirone’s metabolites for D3R relative to those of the parent compound offsets the slower brain delivery of buspirone with oral administration.

Significant inhibition of [11C]raclopride binding in the dorsal striatum following i.m. buspirone indicates that buspirone occupies D3R. Buspirone has been known to possess D2R antagonist properties (Riblet et al., 1982; Dhavalshankh et al., 2007; Bergman et al., 2013). This could be of potential concern when assessing buspirone for the treatment of addiction, which is characterized by decreases in striatal D2R (Volkow et al., 1990, 1993, 1996, 1997; Martinez et al., 2004). Moreover, reduced D3R in striatum is linked with impairment in function of the frontal cortex, which is necessary for self-control, in addicted individuals (Volkow et al., 1993, 2001, 2007) and in animal models, reduced signalling through D2R facilitates compulsive drug use (Everitt et al., 2008). However, this should not be a concern with the use of oral buspirone. Indeed, the minimal D2R occupancy by oral buspirone could explain why buspirone is well tolerated in patients with Parkinson’s disease (Bonifati et al., 1994), in whom it does not produce extra-pyramidal side effects when used to treat anxiety. Furthermore, buspirone was discontinued from development as an anti-psychotic drug (Sathananthan et al., 1975), consistent with minimal effects as a D2R antagonist when administrated orally. In this respect, buspirone may also be of value as a treatment for impulse control disorders that can occur in patients with Parkinson’s disease, particularly with the use of high affinity D3R and D2R agonists (Vilas et al., 2012). Indeed, polymorphisms in the genes encoding for D2R and D3R are implicated in the susceptibility to impulse control disorders (Lee et al., 2009; Eisenegger et al., 2010).

In this study we evaluated parenteral and oral routes of administration, and used a range of doses that would be expected to cover those used therapeutically. However, dose extrapolation across routes of administration and species is potentially problematic; the i.m. doses of buspirone used (0.19 and 0.5 mg/kg) and the oral doses used (1–3 mg/kg) are likely to be at the upper range of what would be routinely administered (60 mg p.o.) for the treatment of anxiety. Nonetheless, these data indicate that therapeutic doses of buspirone in humans will result in significant blockade of D3R (and also, by inference, D2R). To the extent that our findings in the non-human primate brain can be extrapolated to the human brain, our findings indicate that doses of 1.6 mg/kg (human equivalent dose for 3 mg/kg baboon dose) may be sufficient to induce >50% D3R occupancy. Taking into account the known fraction of [11C]PHNO binding to D3R in GP.
CNS conditions that target D3R (and/or D4R). Clinical studies that assess the relationship between plasma concentration of buspirone and its metabolites and the occupancy of D3R may help optimize dosing regimes. Oral buspirone in humans has a very wide safety margin (up to gram levels per day were administered in antipsychotic trials) (Sathananthan et al., 1975). Therefore, higher doses of oral buspirone could be used for clinical translation of the current findings.

Our findings have implications for clinical research examining the efficacy of buspirone for the treatment of SUD. Specifically, the short lived blockade of D3R after oral buspirone indicates that clinical trials will require the use of significantly higher doses (at least three-fold higher) than those used currently to treat anxiety (60 mg) in order to achieve sustained high levels of D3R blockade. Also the difference in the pattern of receptor occupancy between oral buspirone (associated with a more selective blockade of D3R) and i.m. buspirone (associated with blockade of both D2R and D3R) highlight the relevance of buspirone metabolites in its pharmacological effects. Indeed, pilot data showing very high levels of D3R blockade after i.m. administration of 6’-hydroxybuspirone in midbrain (though not in pallidum) suggest that this metabolite warrants further investigation for its potential value in addiction treatment. The short duration of D3R occupancy also suggests that extended release formulations (including those of 6’-hydroxybuspirone) merit investigation for the treatment of SUD or that of other CNS conditions that target D3R (and/or D2R).

**Study limitations and other considerations**

The attribution of buspirone-induced changes in [11C]PHNO and [11C]raclopride binding to D2R and D3R is complicated by the following factors: (1) [11C]PHNO binds to both D2R and D3R in different ratios in different brain regions (Rabiner et al., 2009), and (2) buspirone increases extracellular dopamine, which may differentially affect the binding of the two radiotracers (Gobert et al., 1999; Kaariainen et al., 2008). Nonetheless, we have observed a remarkable decrease in [11C]PHNO BPND in D3R rich regions with i.m. oral buspirone, which is consistent with significant D3R blockade and a significant difference between i.m. and oral buspirone on its effects on [11C]raclopride, which is consistent with significant blockade of D2R by i.m. but not oral buspirone. For oral buspirone, the large reduction in [11C]PHNO binding exceeds the reported percentage of D3R (8%) in PU (Rabiner et al., 2009), which we attribute to both a small degree of D2R binding but also competition from endogenous dopamine released by buspirone (Gobert et al., 1999; Kaariainen et al., 2008). We also cannot exclude underestimation of D3R occupancy due to the presence of D3R in CB (Ginovart et al., 2007; Rabiner et al., 2009), which could affect occupancy measures when using [11C]PHNO (Searle et al., 2013). In addition, unavoidable ‘cold mass’ in the [11C]PHNO preparation (non-tracer dose) can introduce errors in the occupancy estimation (Searle et al., 2013). Due to the slow wash-out properties of [11C]PHNO, a scanning period longer than 90 min would be better for accurate quantification (Girgis et al., 2011) though this would introduce errors due to low counting statistics. Though we cannot exclude the possibility that anaesthesia may have affected radiotracer binding, we controlled the time interval between anaesthesia induction and radiotracer administration and performed all [11C]PHNO studies on separate days to minimize carry-over effects from PHNO on subsequent [11C]PHNO studies. Plasma analysis would have helped us elucidate the contribution of buspirone’s metabolites in D3R occupancies and further studies would have enabled us to estimate the duration of D3R occupancies and to measure D3R occupancies after 5-hydroxybuspirone and 6’-hydroxybuspirone but the closure of the brain imaging laboratory at BNL precluded us from carrying them out. Finally, since the effects on cocaine self-administration were obtained following parenteral administration of buspirone, and we showed that i.m. buspirone occupies both D2 and D3 receptors, we cannot impute the relative importance of each receptor to the effects on cocaine self-administration.

In summary, present findings demonstrate that parenteral buspirone induced a significant occupancy of D2R and D3R, whereas oral administration induced blockade mainly of D3R. Our results suggest that higher doses of oral buspirone than those that are typically used for the treatment of anxiety (at least three-fold higher) will be required in order to achieve and sustain the levels of D3R occupancies reported (Mugnaini et al., 2013) to attenuate drug craving in smokers.

**Supplementary material**

For supplementary material accompanying this paper, visit http://dx.doi.org/10.1017/S1461145714000194

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**References**


