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6-OH Buspirone is a Major Active Metabolite of Buspirone: Assessment of Pharmacokinetics and 5-HT$_{1A}$ Receptor Occupancy in Rats

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List of abbreviations: 1-PP: 1-(2-pyrimidinyl)-piperazine; AUC: Area under the plasma-concentration time profile; C$_{max}$: maximum observed concentration achieved following oral dosing; Cl: plasma clearance; Css: steady-state plasma concentrations; EC$_{50}$: concentration required to reach 50% receptor occupancy; F: oral bioavailability; ia: intra-arterial; po: oral; t$_{1/2}$: half-life; t$_{max}$: time at which C$_{max}$ is observed; Vss: Volume of distribution at steady-state.

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ABSTRACT

The pharmacokinetics and in vivo potency of 6-OH buspirone, a major metabolite of buspirone, was investigated. The plasma clearance (47.3 ± 3.5 mL/min/kg), volume of distribution (2.6 ± 0.3 L/kg), and half-life (1.2 ± 0.2 h) of 6-OH buspirone in rats was similar to that of buspirone. Bioavailability was higher for 6-OH buspirone (19%) when compared to buspirone (1.4%). Following intravenous infusions to steady-state levels in plasma, 6-OH buspirone and buspirone increased 5-HT$_{1A}$ receptor occupancy in a concentration dependent manner with EC$_{50}$ values of 1.0 ± 0.3 µM and 0.38 ± 0.06 µM in the dorsal raphe and 4.0 ± 0.6 µM and 1.5 ± 0.3 µM in the hippocampus, respectively. Both compounds appeared to be ~4-fold more potent in occupying presynaptic 5-HT$_{1A}$ receptors in the dorsal raphe than the postsynaptic receptors in the hippocampus. Oral dosing of buspirone in rats resulted in exposures (area under the concentration-time profile) of 6-OH buspirone and 1-(2-pyrimidinyl)-piperazine (1-PP), another major metabolite of buspirone, that were ~ 12 (6-OH buspirone) and 49 (1-PP) fold higher than the exposure of the parent compound. As a whole, these preclinical data suggest that 6-OH buspirone likely contributes to the clinical efficacy of buspirone as an anxiolytic agent.

INTRODUCTION

Buspirone (Figure 1) is a potent and selective 5-HT$_{1A}$ receptor partial agonist which has been prescribed for the treatment of Generalized Anxiety Disorders (Sramek et al., 2002, Blier and Ward, 2003, Goodman, 2004). Although the onset of buspirone action is relatively slow and the efficacy is only obtained after chronic treatment, therapy with buspirone is not associated with undesirable side effects such as sedation, cognitive impairment, withdrawal symptoms and potential abuse liability that can occur with benzodiazepine treatment (Eison and Temple, 1986, Tunnicliff, 1991, Argyropoulos and Nutt, 1999). Buspirone has also been implicated to have a role in the treatment of depressive disorders (Thase et al., 1998, Blier and Ward, 2003).

Current hypotheses on buspirone's clinical mechanisms of action focus on its agonist activities on 5-HT$_{1A}$ receptors (Schreiber and De Vry, 1993; Blier and Ward, 2003). Belonging to the superfamily of G-protein-coupled receptors, 5-HT$_{1A}$ receptors share a high identity (89%) of their transmembrane-spanning amino acid sequences in human and rats (Albert et al., 1990). They are abundant as presynaptic (somotodendritic) autoreceptors on serotonergic neurons, primarily, in the midbrain dorsal raphe nucleus; activation of the 5-HT$_{1A}$ autoreceptors inhibits the neuronal activity and results in a reduction of 5-HT release in terminal synapses of the serotonergic neurons (Blier and Ward, 2003). 5-HT$_{1A}$ receptors are also present as postsynaptic receptors in the forebrain limbic structures; activation of these receptors inhibits activities of postsynaptic neurons innervated by serotonergic axonal terminals. It has been hypothesized that prolonged activation of 5-HT$_{1A}$ autoreceptors in the dorsal raphe with 5-HT$_{1A}$ agonists, such as

buspirone and its analogues, results in desensitization of the receptors and consequently

increases releases of 5-HT in the limbic regions (Blier and Ward, 2003). The enhanced

serotonergic functions are believed to be responsible for anxiolytic and antidepressant

effects of these agents. While behavioral/clinical effects of buspirone and other 5-HT$_{1A}$

agonists are believed to be mediated through occupying and acting on 5-HT$_{1A}$ receptors,

the level of 5-HT$_{1A}$ receptor occupancy required for the effects is not well investigated.

In two human PET studies, little or no occupancy of 5-HT$_{1A}$ receptors has been observed

following administration of clinically efficacious doses of buspirone (Rabiner et al.,

2000, Passchier et al., 2001). Preclinically, the requirement of 5-HT$_{1A}$ occupancy for

buspirone and its analogues at behaviorally active doses has not been investigated.

In humans and rats, buspirone is extensively metabolized and has low oral

bioavailability (<5%) (Caccia et al., 1983, Jajoo et al., 1989). The metabolic disposition

is similar in the two species with three major metabolic pathways being N-dealkylation to

1-(2-pyrimidinyl)-piperazine (1-PP) and hydroxylation to either 5-hydroxy-buspirone or

6-hydroxy-buspirone (6-OH buspirone, Fig. 1). Of these metabolites, 1-PP has been the

most extensively investigated in its role as an active metabolite (Caccia et al.,1986,

Zuideveld et al., 2002). 1-PP behaves as an α2-adrenoceptor antagonist with a low

affinity to 5-HT$_{1A}$ receptor (Cacia et al., 1986, Gobbi et al., 1991, Gobert et al., 1995) and

therefore is unlikely to play an important role in the anxiolytic effects of buspirone.

Much less is known about the pharmacological properties of 6-OH buspirone.

Conversion to 6-OH buspirone has been shown to be the predominant metabolic pathway

involved in buspirone elimination in human liver microsomes (Zhu et al., 2005). In

addition, plasma levels of 6-OH buspirone have been recently reported to be 40-fold

greater than those of buspirone following oral administration to humans (Dockens et al., 2006). More recently, 6-OH buspirone has been found to possess anxiolytic activity in rats using the fear-induced ultrasonic vocalization paradigm (unpublished observations, Stark AD et al.). The primary aims of the present study are 1) to evaluate the pharmacokinetics of 6-OH buspirone in rats, 2) to characterize the in vivo potency of 6-OH buspirone and buspirone at the 5-HT$_{1A}$ receptor by measuring receptor occupancy using in vivo autoradiography, and 3) to investigate the requirement of 5-HT$_{1A}$ occupancy for buspirone at behaviorally active doses.

METHODS

Pharmacokinetic Studies in Rats

Male Sprague-Dawley rats (weighting 250-350 g) (Charles River, Wilmington, MA) with single carotid artery-catheterization were utilized in this study. Rats (n=3 /dose group) received either a single 5 mg/kg intra-arterial (ia) dose or a single 10 mg/kg oral (po) dose of 6-OH buspirone or buspirone. The vehicle for both the ia and po dosing was 0.2 M sodium acetate buffer, pH 4. Serial blood samples (0.25 ml) were collected at pre-dose, 0.1, 0.2, 0.25, 0.5, 1, 2, 4, 6, 8 and 12 hrs following ia dosing, and at pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8 and 12 hrs following po dosing. Immediately upon collection, the blood was mixed with K_3EDTA and stored on ice. Within 60 min, blood samples were centrifuged at approximately 1000 x g for 15 min at 4 ^{o}C, and plasma was harvested. The plasma samples were stored at -70 ^{o}C until use.

Concentrations of buspirone, 6-OH buspirone and 1-PP in plasma were quantitated using a liquid chromatography tandem mass spectrometric method (LC/MS/MS). Briefly, 50 µL plasma, 50 µl of 10 ng/mL internal standard solution, and 0.2 ml of phosphate buffered saline were mixed. Samples were passed through a conditioned C18 (EC) SPE cartridge, washed with 1 mL water and 0.5 mL of 50/50 (v/v) methanol/water, and eluted with approximately 2 mL of 3% ammonium hydroxide in acetonitrile. The eluted sample was transferred and evaporated to dryness under nitrogen at 40°C. Residues were reconstituted with 0.1 ml of 10/1 (v/v) 100 mM ammonium acetate/ethanol and 10 µL was analyzed using LC/MS/MS. HPLC separation was

achieved using a mobile phase consisting of 50% A: aqueous 5 mM ammonium acetate
(0.1% formic acid), and 50% B: 90:10 methanol:water 5 mM ammonium acetate (0.1%
formic acid) on a Betasil-C18 column (2 x 100 mm, 5μm) (Thermo Scientific, Waltham,
MA) at a flow rate of 250 μl/min with an analysis time of 4 min. Detection was
performed in positive, MRM mode using a Micromass Quattro LC (Waters Corporation,
Milford, MA) with an EI source as the LC/MS/MS interface.

In vivo autoradiography studies:

Male Sprague Dawley rats (weighting 250-350 g) with dual jugular vein-catheterization
(Charles River, Wilmington, MA) were used in this study. Rats were housed in
polycarbonate cages and maintained on a 12:12 hour light dark cycle with free access to
standard chow and water. Buspirone (Bristol-Myers Squibb, Wallingford, CT), 6-OH
buspirone (Bristol-Myers Squibb, Wallingford, CT) and [^{3}H]WAY100635 (a selective 5-
HT_{1A} antagonist, Amersham, NJ, USA) dosing solutions were freshly prepared using
sterile saline as vehicle. For buspirone experiments, rats were intravenously injected with
a loading dose of buspirone (0.45 – 12 mg/kg), immediately followed by a continuous
infusion of the buspirone at 0.45 – 12 mg/kg/hr for 90 min through one jugular vein. For
6-OH buspirone experiments, rats were intravenously injected with a loading dose of 6-
OH buspirone (2.4-23.7 mg/kg), immediately followed by a continuous infusion of 6-OH
buspirone at 2.4-23.7 mg/kg/hr for 90 min through one jugular vein. For both groups of
rats, blood samples (0.3-0.4 ml) were taken at 0, 40, 50 and 60 min post-dose through the
second jugular vein. In previous pilot experiments, it was determined that steady-state

plasma concentrations of buspirone and 6-OH buspirone were achieved by 40 minutes

after the start of the infusion under the described protocol (data not shown). Steady-state

plasma concentrations (Css) of buspirone and 6-OH buspirone are presented as the mean

± SD of the 40, 50 and 60 min timepoints.

Immediately after the last blood sample, 10μCi/100 g body weight

[^{3}H]WAY100635 (in 0.6-0.7 ml saline) was intravenously injected. Rats were decapitated

30 min later, and the brains were collected, frozen and sectioned (20 μm) using a

Cryostat, and sections were mounted on super-frost slides (VWR International,

Wilmington, DE). Brain sections were exposed to tritium-sensitive phosphor screens

(PerkinElmer Life Sciences, Shelton, CT) for 2-3 weeks and images of [^{3}H]WAY100635

binding in the brain were captured and analyzed using Cyclone Storage Phosphor

Imaging System (PerkinElmer Life Sciences, Shelton, CT). The cerebellum, where 5-

HT$_{1A}$ receptor density is nominal, was used as a reference region for defining non-

specific binding. The percent occupancy at 5-HT$_{1A}$ receptors in the region of interest was

calculated as 100% - %([^{3}H]WAY100635 binding in drug-treated - [^{3}H]WAY100635

binding in cerebellum) / ([^{3}H]WAY100635 binding in vehicle - [^{3}H]WAY100635

binding in cerebellum).

Concentrations of buspirone and 6-OH buspirone in the plasma were quantitated

using a liquid chromatography tandem mass spectrometric method (LC/MS/MS).

Briefly, a 0.1 ml of plasma, 50 μl of 200 nM internal standard solution, and 0.1 ml of 0.1

M Na$_2$CO$_3$ were mixed followed by the addition of 1.0 ml of 1:1 MTBE:EtOAc.

Samples were vortexed, centrifuged, and the organic layer was transferred and

evaporated to dryness under nitrogen at 60°C. Residues were reconstituted with 0.1 ml of

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H_2O/CH_3CN/HCOOH: 50/50/0.1 (v/v/v). HPLC separation was achieved using an

acetonitrile (0.1% formic acid) / water (0.1% formic acid) gradient on a Zorbax, SB-C18

column (2 x 50 mm, 5μm) (Agilent Technologies, Palo Alto, CA) at a flow rate of 200

μl/min with an analysis time of 5 min. Detection was performed in positive, MRM mode

using a Micromass Quattro Ultima (Waters Corporation, Milford, MA) with an EI source

as the LC/MS/MS interface.

Data Analysis

Plots of $5HT_{1A}$ receptor occupancy versus plasma concentration were fitted to a one-site

binding model using nonlinear regression according to the following equation:

%Occupancy = Bmax * C/ (EC_{50} + C) where Bmax is the maximal binding, C is the drug

concentration, and EC_{50} is the concentration required for 50% receptor occupancy.

Nonlinear regression was performed using GraphPad Prism version 3.00 (GraphPad

Software, San Diego, CA). Estimates of EC_{50} are reported as the estimate ± SE.

All pharmacokinetic parameters were calculated by noncompartmental methods as

described by Gilbaldi and Perrier (1982). Pharmacokinetic parameters (aside from t_{max})

are reported as the mean ± SD. t_{max} is presented as the median along with the observed

range in parentheses.

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RESULTS

The pharmacokinetic parameters of 6-OH buspirone and buspirone in rats are summarized in Table 1. Plasma clearances for both buspirone and 6-OH buspirone were high in relation to liver blood flow in rats being 68.7 ± 8.7 and 47.3 ± 3.5 mL/min/kg, respectively (Table 1). Estimates of volume of distribution at steady-state (Vss), and half-life $(t_{1/2})$ were also were comparable. 6-OH buspirone had greater exposure following oral administration than buspirone with higher estimates of both bioavailability and C_{max}. (Table 1). Figure 2 shows plasma concentration-time profiles of buspirone, 6-OH buspirone, and 1-PP in rats following a single 5 mg/kg ia or 10 mg/kg po dose of buspirone. Plasma concentrations of both 6-OH buspirone and 1-PP were noticeably higher than that of the parent compound when buspirone was administered orally. Mean area under the concentration-time profile (AUC) estimates for the buspirone metabolites, were approximately 12 (6-OH buspirone) and 48 (1-PP) fold higher than AUC estimates for the parent following oral dosing (Figure 2, Table 2).

The distribution pattern of 5-HT$_{1A}$ receptors labeled by intravenous injections of [3H]WAY100635 in the rat brain is consistent with that reported previously (Hume et al., 1994; Khawaja, 1995). A high density of [3H]WAY100635 binding appeared in the cortex, septum, hippocampus, hypothalamus, and raphe nuclei in the brainstem. An intravenous infusion of buspirone or 6-OH buspirone inhibited [^{3}H]WAY100635 in all these regions in a dose-dependent manner. Figure 3 exhibits representative autoradiograms of the inhibitory effect of 6-OH buspirone on in vivo [^{3}H]WAY100635

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binding in the forebrain areas including the hippocampus from rats receiving various doses of 6-OH buspirone (Figure 3A, B, C).

The percentage of $5\text{-}HT_{1A}$ receptor occupancy in two representative brain regions, the hippocampus and the dorsal raphe nucleus, steady-state plasma concentrations, and infusion rates for buspirone and 6-OH buspirone are summarized in Tables 3 and 4. In rats dosed *via* an intravenous infusion with buspirone, 6-OH buspirone Css were ~6-20 fold less than buspirone Css. The range of buspirone and 6-OH buspirone infusion rates resulted in a wide range of steady-state concentrations. Occupancy of $5\text{-}HT_{1A}$ receptors increased with increasing steady-state concentrations of both compounds.

Figure 4 examines the relationship between brain $5\text{-}HT_{1A}$ receptor occupancy in hippocampus and dorsal raphe, and plasma concentrations of buspirone and 6-OH buspirone. The data for each brain region was fitted to a one-site binding model and the estimated in vivo EC_{50} values (plasma concentrations at which 50% $5\text{-}HT_{1A}$ receptor occupancy occurs) are presented in Table 5. The in vivo affinity of buspirone (or its metabolites) appeared more potent for $5\text{-}HT_{1A}$ receptors in the dorsal raphe than in the hippocampus (Table 5, Figure 4). Likewise, 6-OH buspirone exhibited a higher in vivo affinity in the dorsal raphe compared to the hippocampus.

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DISCUSSION

The clearance of buspirone in rats estimated from the current study is similar to the

clearance estimate of 51 ml/min/kg observed by Caccia et al. (1983). 6-OH buspirone

showed similar pharmacokinetics as buspirone in rats with the exception of mean oral

bioavailability which was ~ 13-fold higher (Table 1). A recent pharmacokinetic study in

humans, reported a similar range of half-lives for both buspirone (2.8 – 4.6 hours) and 6-

OH buspirone (4.7 – 4.3 hours) following oral administration of buspirone which is

consistent with similar elimination characteristics of both compounds in humans

(Dockens et al., 2006). Currently, there is no literature information on the oral

bioavailability of 6-OH buspirone in humans.

The *in vivo* affinity/ potency of buspirone and 6-OH buspirone at $5\text{-}HT_{1A}$

receptors in the brain was examined following intravenous infusions of both compounds

to steady-state in the rat. In vivo binding of $[^3H]WAY100635$, a selective $5\text{-}HT_{1A}$

antagonist, in the hippocampus and dorsal raphe was dose-dependently inhibited by

buspirone and 6-OH buspirone, indicating their interaction with $5\text{-}HT_{1A}$ receptors in vivo.

The in vivo affinity at $5\text{-}HT_{1A}$ receptors for both compounds was 3-4 fold higher in the

dorsal raphe than in the hippocampus (Table 5). As elucidated in the Introduction, the

forebrain regions including the hippocampus express postsynaptic $5\text{-}HT_{1A}$ receptors,

whereas the dorsal raphe posses $5\text{-}HT_{1A}$ somatodendritic autoreceptors. A higher affinity

to the autoreceptors relative to the postsynaptic receptors has been observed with

pindolol, a β-adrenoreceptor antagonist with a high $5\text{-}HT_{1A}$ affinity, and been postulated

to underlie the effect of pindolol for augmentation of SSRI antidepressant efficacy

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(Raurich et al., 1999, Martinez et al., 2001, Rabiner et al, 2000). The dorsal raphe has also been considered to play an important in anxiolytic effects of 5-HT$_{1A}$ partial agonists including buspirone whose effects has been hypothesized to be mediated by desensitizing 5-HT$_{1A}$ autoreceptors in the dorsal raphe (Sim-Selley et al., 2000). Our observation of a higher affinity binding of buspirone and its major metabolite, 6-OH buspirone, to 5-HT$_{1A}$ autoreceptors in the dorsal raphe supports the hypothesis.

Although the 6-OH buspirone was present in the plasma following buspirone infusions, the levels of the metabolite were 6-20 fold less than the parent compound. As the *in vivo* affinity of 6-OH buspirone at the 5-HT$_{1A}$ receptor is comparable to buspirone (Table 5), the contribution of this metabolite in occupying 5-HT$_{1A}$ receptors in the buspirone experiments is likely low. Although 1-PP was not quantitated in this experiment, it has been shown to have a low *in vitro* affinity and selectivity at 5-HT$_1$ receptors in rat brain (Caccia et al., 1986; Gobbi et al., 1991), and thus is not likely to contribute significantly to the 5-HT$_{1A}$ occupancy observed in our studies.

To our knowledge, this is the first published report on the activity of 6-OH buspirone at the 5-HT$_{1A}$ receptor. Based upon the current study, the *in vivo* affinity/potency of 6-OH buspirone appears to be comparable to that of buspirone. Buspirone has been shown to have extensive hepatic first pass metabolism in both rats and humans with a reported bioavailability of 4% in humans (Caccia et al., 1983; Mahmood and Sahajwalla, 1999). Although we observed relatively low levels of 6-OH buspirone compared to buspirone following intravenous infusions (Table 3) and intra-arterial dosing (Table 2) of the parent compound, it could contribute significantly to the biological activity of buspirone following oral dosing due to the higher circulating levels

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of metabolites resulting from extensive first pass metabolism. The current

pharmacokinetic study in rats show oral exposures of 6-OH buspirone that are ~ 12 fold

higher than buspirone following a 10 mg/kg oral buspirone dose (Figure 2, Table 2). In

humans, oral exposures of 6-OH buspirone are even higher being ~ 40 fold greater than

buspirone when given oral doses of buspirone over the therapeutic dose range (10 to 60

mg daily) (Dockens et. al., 2006).

Receptor occupancy requirements associated with an anxiolytic effect at the 5-

HT_{1A} receptor is not well understood. In both the fear induced and air-puff elicited

ultrasonic vocalization models of anxiety in rats, buspirone has been shown to have

anxiolytic effects at an oral dose of 10 mg/kg (Vis et al., 2001; Naito et al., 2003). Based

upon concentrations of buspirone and 6-OH buspirone observed in the current study and

in vivo EC_{50} estimates presented in Table 5, maximum 5-HT_{1A} receptor occupancies of

~23% in the dorsal raphe and ~7% in the hippocampus occur following an oral dose of 10

mg/kg to rats. In a recent study in humans, buspirone and 6-OH buspirone concentrations

were monitored following 5 days of buspirone administration over the therapeutic dose

range (Dockens et al., 2006). At the highest dose used in the study (i.e. oral doses of 30

mg/kg twice daily), mean C_{max} values of 2.0 ng/mL (0.005 µM) and 39 ng/mL (0.097

µM) were reported for buspirone and 6-OH buspirone, respectively. These

concentrations would result in 5-HT_{1A} receptor occupancies of ~10% in the dorsal raphe

and ~3% in the hippocampus using in vivo EC_{50} estimates presented in Table 5.

Low levels of 5-HT_{1A} receptor occupancy by buspirone at clinically effective

doses have been reported previously in human PET studies (Rabiner et al., 2000,

Passchier et al 2001). A single dose of 10 mg or 40 mg of buspirone occupies ~5% 5-

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HT$_{1A}$ receptors in healthy human subjects (Rabiner et al., 2000, Passchier et al 2001). A similar low fraction (<10%) of occupancy in humans has been observed with other selective and nonselective 5-HT$_{1A}$ agonists such as tandospirone, flesinoxen and EMD 128 130 following single or multiple clinical doses capable of activating central 5-HT$_{1A}$ receptor functions (Rabiner et al., 2002, Nakayama et al., 2002, Bantick et al., 2004). Results of the current study are consistent with these literature observations suggesting that high levels of 5-HT$_{1A}$ receptor occupancy are not required to elicit an anxiolytic effect both preclinically in rats and clinically in humans.

In summary, the present study demonstrates that 6-OH buspirone is the major active metabolite of buspirone with similar in vivo potency at the 5-HT$_{1A}$ receptor. 6-OH buspirone has improved oral exposure in comparison to buspirone and could be an effective anxiolytic agent alternative to buspirone. Finally, results of our current study are consistent with literature reports that suggest a low 5-HT$_{1A}$ receptor occupancy requirement is needed for anxiolytic activity.

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FOOTNOTES

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LEGENDS FOR FIGURES

Figure 1   Chemical structure of buspirone and 6-OH buspirone

Figure 2   Plasma-concentration time profile of buspirone and its metabolites, 6-OH
buspirone and 1-PP, following (A) intra-arterial (5 mg/kg) and (B) oral (10
mg/kg) administration of buspirone

Figure 3   Inhibition of [^{3}H]WAY100635 binding in rat brain following intravenous
infusions of 6-OH buspirone (A, B, C) at various infusion rates. A: vehicle; B:
2.4 mg/kg/hr; C: 11.9 mg/kg/hr. Abbreviation: Hip: hippocampus. Bar scale, 2
mm.

Figure 4   Relationship between 5-HT$_{1A}$ receptor occupancy in two brain regions
(hippocampus and dorsal raphe) and plasma concentration following
intravenous infusions of buspirone (A) and 6-OH buspirone (B).

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Table 1 Pharmacokinetics of 6-OH buspirone and buspirone in male SD rats (n=3 per dose route) following single intra-arterial (IA) or oral (PO) administration.

	6-OH Buspirone		**Buspirone**	
Dose (mg/kg)	5	10	5	10
Route	IA	PO	IA	PO
Cl (mL/min/kg)	47.3 ± 3.5	-	68.7 ± 8.7	-
Vss (L/kg)	2.6 ± 0.3	-	2.5 ± 0.3	-
$t_{1/2}$ (h)	1.2 ± 0.2	1.2 ± 0.3	0.9 ± 0.4	1.0 ± 0.0
C_{max} (µM)	-	0.59 ± 0.16	-	0.04 ± 0.02
t_{max} (h)[a]	-	0.25 (0.25 -4.0)	-	4.0 (0.25-4.0)
F (%)	-	19.1 ± 9.1	-	1.4 ± 0.4

[a] t_{max} presented as median followed by the observed range in parentheses.

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Table 2 Exposures of buspirone, 6-OH buspirone, and 1-PP in male SD rats following single intra-arterial (IA) or oral (PO) administration of buspirone.

	Buspirone	6-OH buspirone	1-PP
Buspirone - 5 mg/kg IA (n=3)			
C_{max} (µM)	5.51 ± 0.65	0.26 ± 0.14	0.69 ± 0.05
AUC (µM*h)	3.18 ± 0.38	0.39 ± 0.18	2.24 ± 0.31
Buspirone - 10 mg/kg PO (n=3)			
C_{max} (µM)	0.04 ± 0.02	0.26 ± 0.06	0.69 ± 0.19
AUC (µM*h)	0.09 ± 0.03	1.09 ± 0.21	4.41 ± 0.56

Table 3 Steady-state concentrations of buspirone and 6-OH buspirone, and 5-HT$_{1A}$ receptor occupancy in the hippocampus and dorsal raphe following intravenous infusions of buspirone.

| Rat ID	Infusion rate (mg/kg/hr)	Buspirone Css (μM)	6-OH Buspirone Css (μM)	% 5-HT$_{1A}$ Receptor Occupancy	
				Hippocampus	Dorsal Raphe
A	12.0	11.1 ± 0.1	1.7 ± 0.3	95	100
B	12.0	12.0 ± 0.5	1.6 ± 0.1	85	100
C	4.0	6.36 ± 1.92	0.29 ± 0.04	72	96
D	1.3	1.27 ± 0.09	0.11 ± 0.01	44	87
E	0.45	0.46 ± 0.12	0.04 ± 0.02	28	53

Table 4   Steady-state concentrations and 5-HT$_{1A}$ receptor occupancy in the hippocampus and dorsal raphe following intravenous infusions of 6-OH buspirone.

Rat ID	Infusion rate (mg/kg/hr)	Css (µM)	% 5-HT$_{1A}$ Receptor Occupancy	
			Dorsal Raphe	Hippocampus
1	23.7	22.4 ± 2.8	90	85
2	23.7	24.1 ± 1.4	96	98
3	23.7	20.0 ± 2.4	93	93
4	11.9	12.1 ± 0.6	77	75
5	11.9	11.0 ± 2.2	86	77
6	11.9	11.2 ± 0.5	85	78
7	2.4	2.3 ± 0.2	55	35
8	2.4	2.9 ± 0.3	78	52
9	2.4	2.7 ± 0.1	74	44

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Table 5 Estimated in vivo EC_{50} of buspirone and 6-OH buspirone to 5-HT_{1A} receptors in the hippocampus and dorsal raphe of the rat.

Compound	In vivo EC_{50} (μM)	
	Hippocampus	**Dorsal Raphe**
Buspirone	1.5 ± 0.3	0.38 ± 0.06
6-OH buspirone	4.0 ± 0.6	1.0 ± 0.3

Figure 1
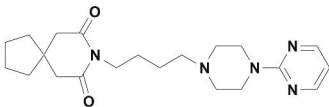
Buspirone
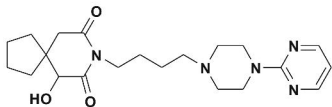
6-OH buspirone

Figure 2

Figure 3

Figure 4