

RESEARCH PAPER

Signalling profile differences: paliperidone versus risperidone

W P Clarke, T A Chavera, M Silva, L C Sullivan and K A Berg

Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX, USA

Correspondence

Kelly A Berg, PhD, Department of Pharmacology – MS 7764, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900, USA. E-mail: berg@uthscsa.edu

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BACKGROUND AND PURPOSE

Paliperidone is an active metabolite of the second-generation atypical antipsychotic, risperidone recently approved for the treatment of schizophrenia and schizoaffective disorder. Because paliperidone differs from risperidone by only a single hydroxyl group, questions have been raised as to whether there are significant differences in the effects elicited between these two drugs.

EXPERIMENTAL APPROACH

We compared the relative efficacies of paliperidone versus risperidone to regulate several cellular signalling pathways coupled to four selected GPCR targets that are important for either therapeutic or adverse effects: human dopamine D₂, human serotonin 2A receptor subtype (5-HT_{2A}), human serotonin 2C receptor subtype and human histamine H₁ receptors.

KEY RESULTS

Whereas the relative efficacies of paliperidone and risperidone were the same for some responses, significant differences were found for several receptor-signalling systems, with paliperidone having greater or less relative efficacy than risperidone depending upon the receptor–response pair. Interestingly, for 5-HT_{2A}-mediated recruitment of β -arrestin, 5-HT_{2A}-mediated sensitization of ERK, and dopamine D₂-mediated sensitization of adenylyl cyclase signalling, both paliperidone and risperidone behaved as agonists.

CONCLUSIONS AND IMPLICATIONS

These results suggest that the single hydroxyl group of paliperidone promotes receptor conformations that can differ from those of risperidone leading to differences in the spectrum of regulation of cellular signal transduction cascades. Such differences in signalling at the cellular level could lead to differences between paliperidone and risperidone in therapeutic efficacy or in the generation of adverse effects.

Abbreviations

AA, arachidonic acid; APD, antipsychotic drug; G protein, guanine nucleotide-binding protein; 5-HT_{2A}, serotonin 2A receptor subtype; 5-HT_{2C}, serotonin 2C receptor subtype

Introduction

Paliperidone is a second-generation antipsychotic drug (APD) recently approved for the treatment of schizophrenia and schizoaffective disorder. In contrast to first-generation (typical) APDs, second-generation APDs have lower risks of extrapyramidal side effects and tardive dyskinesia and are

referred to as ‘atypical’ APDs. In addition, there is some evidence, although controversial, that the atypical APDs may have improved efficacy for the treatment of cognition and some of the negative symptoms of schizophrenia, such as apathy, anhedonia and social withdrawal. However, there is a higher risk of weight gain and metabolic syndrome with atypical APD use. Although antagonism or weak efficacy at

dopamine D₂ receptors appears essential for antipsychotic activity, the complete molecular mechanism that underlies the therapeutic efficacy of atypical APDs is still unknown. In recent years, several theories have been postulated to account for atypicality, including that atypical APDs have (i) higher affinity for serotonin 2A receptor subtype (5-HT_{2A}) receptors than for dopamine D₂ receptors (Meltzer hypothesis), (ii) faster off-rate of binding to D₂ receptors and (iii) agonism at presynaptic versus antagonism at post-synaptic D₂ receptors (as seen with aripiprazole; for a review, see MacDonald and Bartolome, 2010). Atypical APDs bind to a variety of receptor subtypes [including dopamine D₂, 5-HT_{2A}, serotonin 2C receptor subtype (5-HT_{2C}) and histamine H₁, among others]. As schizophrenia is a multidimensional disease likely with several aetiologies and pathologies, action at a variety of receptor targets may be an important characteristic for effective therapeutic treatment (Gray and Roth, 2007; Wong *et al.*, 2008).

Paliperidone is the major active metabolite of another, well utilized, atypical APD, risperidone, differing by only a single hydroxyl group. With such high structural similarity, it is reasonable to question whether treatment with paliperidone differs substantively from that of treatment with risperidone (Citrome, 2007; 'Paliperidone: new drug', 2007; Dolder *et al.*, 2008; Dopheide, 2008). Clinical differences between paliperidone versus risperidone may stem from different pharmacokinetic properties and different formulations (extended release vs. oral). Since paliperidone and risperidone display simple, competitive antagonism in radioligand-binding assays with similar affinities for their target receptors (Schotte *et al.*, 1996; Gray and Roth, 2007; Dolder *et al.*, 2008), differences in therapeutic efficacy may result from differences in their ability to regulate receptor-mediated signalling pathways.

It is now well accepted that receptors activate cellular signalling cascades in the absence of a stimulating ligand (agonist) and that this 'constitutive' receptor activity can be decreased by inverse agonists. Although risperidone is known to behave as a competitive antagonist, it also has inverse agonist properties at D₂, 5-HT_{2A}, 5-HT_{2C} and H₁ receptors (Egan *et al.*, 1998; Rauser *et al.*, 2001; Weiner *et al.*, 2001; Akam and Strange, 2004). While the inverse agonist efficacy of risperidone has been well studied, by contrast, there is little known about the efficacy characteristics of paliperidone.

Individual receptor subtypes can regulate the activity of several signal transduction pathways in cells (Perez and Karnik, 2005). A large body of experimental evidence has accumulated recently to suggest that different drugs, acting at a single receptor subtype, can elicit different profiles of signal transduction pathway regulation. This process has been termed 'functional selectivity' or 'biased agonism' (for reviews see Clarke, 2005; Kenakin, 2007; 2011; Urban *et al.*, 2007). The mechanism underlying functional selectivity is based on the production of ligand-dependent conformational states of the receptor, which have differential ability to interact with and regulate each of the multiple signal transduction molecules [i.e. guanine nucleotide-binding protein (G proteins), β -arrestins, etc.], which couple to the receptor. Thus, drugs have selectivity that extends beyond that afforded by differential affinity for different receptor subtypes. Both agonists and inverse agonists can selectively regulate the activity

of distinct signalling pathways coupled to a receptor (Clarke, 2005; Kenakin, 2007; Urban *et al.*, 2007; Kenakin, 2011). It has been suggested that differences in functional selectivity may underlie differences in therapeutic efficacy and/or adverse effect liability of APDs (Heusler *et al.*, 2007).

Importantly, small differences in molecular structure between drugs can lead to pronounced differences in functional selectivity, even for drugs that have similar affinities for a receptor (Moya *et al.*, 2007). Thus, it is possible that even though paliperidone differs from risperidone by a single hydroxyl group and have similar binding affinities for many receptors, they may differ in the profile of cellular signalling pathways they regulate and thus in their therapeutic efficacy or adverse effect profile. In this regard, it is useful to consider that the difference between dopamine and norepinephrine, two drugs with markedly different functional actions, is a single hydroxyl group. Interestingly, risperidone and paliperidone have been found to differ in their effects on the firing rate of serotonergic and noradrenergic neurons *in vivo* (Dremencov *et al.*, 2007).

The goal of this work was to examine differences in the signalling profiles of paliperidone versus risperidone for each of several cellular responses coupled to a selected group of target receptors that are important in therapeutic action or in the production of adverse effects. We have found and report here that the single hydroxyl group of paliperidone does indeed confer differences in the cellular signalling profiles in comparison to that of risperidone.

Methods

Materials

The following compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA): DOI, SB 206553, ketanserin, quinirole, butaclamol, mepyramine and histamine. *myo*-[³H]-inositol and [³H]-arachidonic acid were purchased from PerkinElmer Life and Analytical Sciences (Boston, MA, USA). Risperidone and paliperidone were provided by Janssen Scientific Affairs, LLC (Titusville, NJ, USA). FBS was from Atlanta Biologicals (Atlanta, GA, USA). All other tissue culture reagents were purchased from Invitrogen (Carlsbad, CA, USA). All other drugs and chemicals (reagent grade) were purchased from Sigma-Aldrich.

Cell culture

5-HT_{2C}-mediated responses were measured in CHO-1C19, CHO-1C7 or U2OS cells that express stably the human 5-HT_{2C} receptor. CHO-1C7 cells express the 5-HT_{2C} receptor at high levels and are optimized to detect inverse agonist properties by measuring a reduction in basal effector activities (Berg *et al.*, 1999; Lopez-Gimenez *et al.*, 2001; Brea *et al.*, 2003). CHO cell lines were maintained in minimum essential medium, α -formulation, supplemented with 5% FBS and 300 μ g·mL⁻¹ hygromycin. U2OS cells (DiscoverRx, Freemont, CA, USA), which stably express the human 5-HT_{2C} receptor tagged with a 42 amino acid fragment of β -galactosidase (ProLink™; DiscoverRx Corporation, Freemont, CA, USA), were used to measure recruitment of β -arrestin to the receptor (see below) were grown according to the manufacturer's protocol.

5-HT_{2A}-mediated responses were measured in CHO-FA4 or HEK-293 cells that stably express the human 5-HT_{2A} receptor (HEK-2A cells). CHO-FA4 cells were maintained as described above for CHO cells. HEK-2A cells were obtained from Bryan Roth (University of North Carolina) and maintained in α -MEM supplemented with 10% FBS. Recruitment of β -arrestin was measured with U2OS cells that stably express the human 5-HT_{2A} receptor tagged with Prolink maintained as described above.

Dopamine D₂-mediated responses were measured in HEK-293 and CHO cells stably expressing the human dopamine D₂ receptor. CHO-D2 cells were maintained in α -MEM supplemented with 5% FBS and 50 $\mu\text{g}\cdot\text{mL}^{-1}$ G418. HEK-D2 cells were maintained in DMEM supplemented with 10% FBS and 50 $\mu\text{g}\cdot\text{mL}^{-1}$ G418. Recruitment of β -arrestin was measured with CHO cells stably expressing the human dopamine D₂ receptor tagged with Prolink.

Histamine H₁-mediated responses were measured in HEK cells stably expressing human H₁ receptors or in CHO cells transiently expressing human histamine H₁ receptors were maintained as described above. For recruitment of β -arrestin, CHO cells expressing the human histamine H₁ receptor tagged with Prolink were used and maintained as described above.

All cells were grown in serum-free media for 24 h before each experiment. Parent (non-transfected) cells for each receptor subtype were screened with the ligands to assess possible off-target or non-specific responses. None of the drugs used here altered signalling in the parent cell lines.

Inositol phosphate (IP) accumulation

Measurements of PLC-mediated IP accumulation in response to agonist or constitutive receptor activity were made after 10 min or 30 min of drug exposure, respectively, as described previously (Berg *et al.*, 1998; 1999; 2001b; 2006; 2008).

Arachidonic acid (AA) release

Measurements of PLA₂-mediated AA release in response to agonist or constitutive receptor activity were made after 10 or 30 min of drug exposure, respectively, as described previously (Berg *et al.*, 1998; 1999; 2001b; 2006; 2008).

cAMP accumulation

Measurement of ligand-induced changes in adenylyl cyclase activity (cAMP accumulation) were done as described previously (Berg *et al.*, 1994; 2011; 2003; 2007; Evans *et al.*, 2001).

ERK activity

Changes in ERK activity were measured as described previously (Berg *et al.*, 2011) using the SureFire phospho-ERK (pERK) assay kit (PerkinElmer) according to the manufacturer's directions. The fluorescence signal from pERK was measured in duplicate using a Fluostar microplate reader (BMG Labtech, Offenburg, Germany) with AlphaScreen settings.

β -arrestin recruitment

The PathHunter[®] β -arrestin GPCR Assay kit (DiscoverRX) was used to measure recruitment of β -arrestin to the receptor according to the manufacturer's directions. Chemiluminescence was read with a Fluostar microplate reader (BMG Labtech).

PLC and ERK sensitization

In the absence of a stimulating ligand, receptor signalling systems can exist in a state of constitutive, partial desensitization as a result of constitutive activation of desensitization signalling pathways (Berg *et al.*, 1999; 2006; Wilbanks *et al.*, 2002). Prolonged (>4 h) treatment with an inverse agonist, which reduces constitutive receptor activity, can promote re-sensitization of the receptor-signalling system that can be visualized by enhanced responsiveness to agonist stimulation. To measure sensitization of receptor-PLC or receptor-ERK signalling pathways, cells were treated for 24 h with ligand or vehicle and washed three times with 5 min incubation intervals at 37°C. Cells were then stimulated with a receptor agonist for 10 min to measure IP accumulation or for 0–30 min to measure ERK activation as described above.

[³H]-raclopride binding

Membranes from CHO cells expressing human dopamine D₂ receptors were prepared as described previously (Berg *et al.*, 1999; 2001a; 2008). After ligand treatment (24 h, 100 \times K_i), cells grown in 10 cm² plates, were washed three times with 5 min incubation intervals at 37°C to remove residual drug. Cells were scraped and membranes prepared by trituration through a 26-gauge needle, followed by centrifugation at 39 000 \times g and sonication at 4°C. Saturation binding assays using [³H]-raclopride in cell membrane preparations (50 μg protein/tube) were done using 15 concentrations of radioligand in duplicate over a 3-log unit range as previously described (Berg *et al.*, 1999; 2001a; 2008). Incubations were carried out for 1 h at 37°C followed by rapid filtration through 0.3% polyethylenamine coated GF/C glass fiber filters. Non-specific binding was determined in the presence of 10 μM spiperone.

Data analysis

Curve fitting. Concentration-response data were fit with non-linear regression to the model

$$E = \frac{E_{\max}}{1 + \left(\frac{EC_{50}}{A}\right)^n}$$

where E is the measured response at a given concentration (A), E_{\max} is the maximal response, EC_{50} is the concentration of agonist producing the half-maximal response, and n is the slope index.

For saturation-binding experiments, data were fit with nonlinear regression to the model:

$$B = \frac{B_{\max}}{1 + (K_d/[D])^n} + m \cdot D$$

where B is the measured amount of radioligand bound (fmol $\cdot\text{mg}^{-1}$ protein) in the presence of various concentrations of radioligand $[D]$, B_{\max} is the maximal amount bound, K_d is the concentration of D that produces half-maximal binding, n is the slope factor and m is the slope of the linear regression line for non-specific binding.

Statistical analysis. Data are presented as mean \pm SEM from at least three independent experiments. Statistical differences in concentration-response curve parameters (i.e. E_{\max}) or in response to maximal concentrations of ligands followed by

Bonferroni's *post hoc* using Prism software (Graphpad Software, Inc., San Diego, CA, USA). $P < 0.05$ was considered statistically significant.

Relative efficacy determinations. The ratio of the E_{\max} of a test drug to the E_{\max} of a reference drug was used to characterize the signalling profile of paliperidone and risperidone (Berg and Clarke, 2006; Clarke and Berg, 2010). Measurement of relative efficacy removes the system dependency and permits assessment of differences in the ability of drugs to regulate receptor activity. The relative efficacies of paliperidone and risperidone for selected cellular signalling responses at human 5-HT_{2A}, 5-HT_{2C}, D₂ and H₁ receptors expressed in CHO or HEK cells were obtained from analysis of concentration-response curves (E_{\max} values) or from measurement of responses to maximal ligands concentrations ($100 \times K_i$), which are expected to produce full receptor occupancy. The reference ligands used were ketanserin, SB 206553, butaclamol and mepyramine for 5-HT_{2A}, 5-HT_{2C}, D₂ and H₁ receptors respectively.

Results

5-HT_{2C} receptor signalling

The degree of ligand-independent 5-HT_{2C} receptor activity can most easily be measured as the magnitude of the reduction in basal effector activity (i.e. PLC, PLA₂) produced by an inverse agonist (Berg *et al.*, 1999). As shown in Figure 1, paliperidone and risperidone were efficacious inverse agonists for reduction in basal IP accumulation in CHO cells expressing stably the human 5-HT_{2C} receptor. The maximal reduction in basal IP accumulation for the reference 5-HT_{2C} inverse agonist, SB 206553, was $72 \pm 6\%$. The maximal reduction in IP accumulation produced by paliperidone ($84 \pm 1\%$) was significantly greater than that for risperidone ($68 \pm 5\%$). Relative efficacy values, using SB 206553 as the reference ligand, were 1.30 ± 0.03 and 1.02 ± 0.05 for paliperidone and risperidone respectively. The potency values (pEC_{50}) for SB 206553, paliperidone and risperidone were 7.77 ± 0.24 (17 nM), 6.75 ± 0.22 (178 nM) and 6.54 ± 0.05 (286 nM) respectively.

Figure 2 shows the effects of maximal concentrations ($100 \times K_i$) of SB 206553 (300 nM), paliperidone (3 μ M) and risperidone (1 μ M) on IP accumulation, AA release, ERK activity and β -arrestin recruitment. As reported previously (Berg *et al.*, 2005; 2008), the level of constitutive 5-HT_{2C} receptor activity differed for different responses as evidenced by different magnitudes of reduction of basal activity by SB 206553. Constitutive activity of the 5-HT_{2C} receptor towards the PLC pathway was considerably greater than that towards AA release, ERK activity and β -arrestin recruitment. For the PLA₂ response, SB 206553 and risperidone were inverse agonists, reducing basal AA release by $28 \pm 1\%$ and $14 \pm 2\%$ respectively. By contrast, paliperidone behaved as an antagonist and did not reduce basal AA release (maximal reduction = $0.5 \pm 8\%$). For the ERK response, all three ligands were inverse agonists with equal efficacy to reduce basal ERK activity by approximately 25%. Similarly, all three ligands were weak inverse agonists with equal efficacy to reduce basal β -arrestin recruitment to the 5-HT_{2C} receptor.

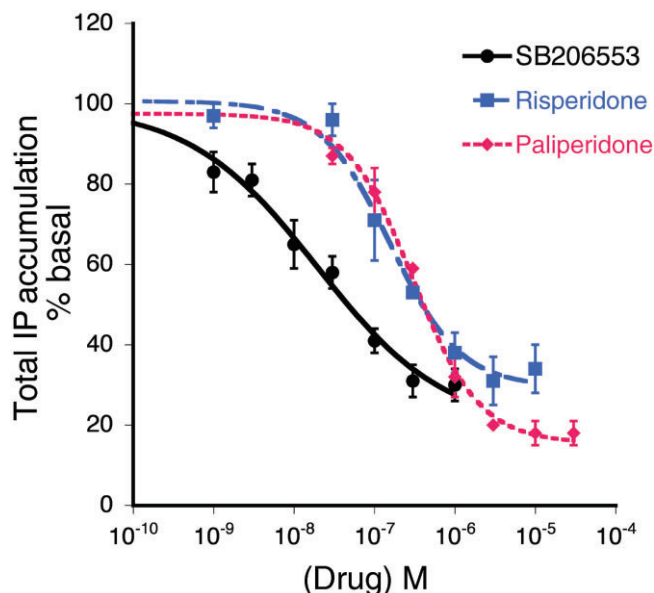


Figure 1

Inverse agonism of risperidone and paliperidone at the 5-HT_{2C} receptor for the PLC-IP pathway. Concentration-response curves to SB 206553 (reference ligand), risperidone and paliperidone for reduction of basal IP accumulation in CHO-1C7 cells expressing the human 5-HT_{2C} receptor. Cells, pre-labelled with [³H]-myo-inositol, were incubated with the indicated concentrations of ligands for 30 min in the presence of LiCl (20 mM). Data are expressed as the percent decrease in basal IP accumulation and represent mean \pm SEM of three experiments. Maximal reduction in IP accumulation produced by SB 206553, risperidone and paliperidone were $72 \pm 6\%$, $68 \pm 5\%$ and $84 \pm 1\%$ respectively. The maximal reduction in basal IP produced by paliperidone was significantly greater than that produced by risperidone ($P < 0.05$, one-way ANOVA followed by Bonferroni's *post hoc* test).

Similarly to agonist stimulation, prolonged ligand-independent receptor activity can lead to a reduction of effector activity (constitutive desensitization; Berg *et al.*, 1999; Marion *et al.*, 2004). Prolonged pretreatment (>4 h) with an inverse agonist can reduce constitutive desensitization of some receptor-signalling systems, which can be visualized as an enhanced response to agonist following washout of the inverse agonist. For the 5-HT_{2C} receptor, prolonged pretreatment (24 h) with SB 206553 enhanced agonist (DOI)-stimulated IP accumulation by approximately 100% and maximal ERK activity by approximately 285% over that of DOI stimulation following vehicle pretreatment (Figure 3). For both responses, the relative efficacy of paliperidone exceeded that of risperidone and for both responses, paliperidone was a more efficacious inverse agonist than was the prototypical 5-HT_{2C} inverse agonist, SB 206553. Prolonged pretreatment with paliperidone or risperidone enhanced DOI-stimulated IP accumulation by 150% [$pEC_{50} = 6.87 \pm 0.42$ (135 nM)] and 85% [$pEC_{50} = 7.94 \pm 0.18$ (12 nM)] respectively (Figure 3A). Following vehicle pretreatment, maximal DOI-stimulated ERK activity (at 2 min) was $201 \pm 17\%$ over basal. Pretreatment with SB 206553, paliperidone and risperidone enhanced DOI-stimulated ERK activity by $380 \pm 36\%$, $433 \pm 12\%$ and $574 \pm 94\%$ respectively (Figure 3B).

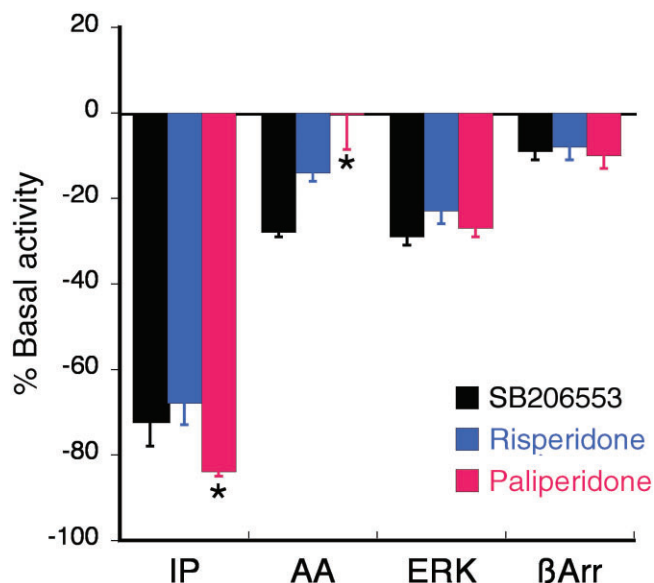


Figure 2

Acute effects of risperidone and paliperidone at the 5-HT_{2C} receptor for various responses compared with the reference ligand, SB 206553. Maximal effects of the ligands on IP accumulation are derived from the E_{\max} values from the concentration-response curves shown in Figure 1. Reduction of basal AA release and ERK activation by maximal concentrations ($100 \times K_i$) of SB 206553 (300 nM), risperidone (1 μ M) and paliperidone (3 μ M) were measured in CHO-1C7 cells following 30 min (AA release) or 5 min (ERK activity) of incubation with ligand. Recruitment of β -arrestin to the 5-HT_{2C} receptor was measured in U2Os cells following incubation with maximal concentration of ligands for 90 min. DOI-mediated data are expressed as the percent reduction in basal activity and bars represent the mean \pm SEM of six experiments. * $P < 0.05$ for the effect of paliperidone compared with that of risperidone (one-way ANOVA followed by Bonferroni's *post hoc* test).

5-HT_{2A} receptor signalling

Figure 4 shows the effect of acute treatment of cells expressing the human 5-HT_{2A} receptor with maximal concentrations ($100 \times K_i$) of paliperidone and risperidone, compared to the reference ligand, ketanserin. For the 5-HT_{2A}-PLC and the 5-HT_{2A}-ERK pathways, all three ligands were inverse agonists of comparable efficacy, reducing basal IP accumulation by $38 \pm 4\%$, $43 \pm 7\%$ and $39 \pm 5\%$ and reducing basal ERK activity by $21 \pm 3\%$, $30 \pm 5\%$ and $30 \pm 4\%$ for ketanserin, risperidone and paliperidone respectively. Only risperidone significantly reduced AA release ($17 \pm 4\%$). Interestingly, all three ligands behaved as weak agonists of comparable efficacy for the recruitment of β -arrestin to the 5-HT_{2A} receptor. Whereas 5-HT (10 μ M) produced a robust β -arrestin translocation of $660 \pm 50\%$ above basal (mean \pm SEM, $n = 6$), ketanserin, risperidone and paliperidone increased β -arrestin recruitment to the 5-HT_{2A} receptor by $177 \pm 29\%$, $197 \pm 23\%$ and $165 \pm 25\%$ above basal respectively.

Prolonged pretreatment with a maximal concentration ($100 \times K_i$) of the reference inverse agonist, ketanserin (1 μ M) increased 5-HT_{2A} agonist (DOI)-stimulated IP accumulation by 28% over that of vehicle pretreatment (Figure 5A). For this response, both risperidone and paliperidone behaved as

inverse agonists of comparable efficacy. Pretreatment with risperidone or paliperidone enhanced DOI-stimulated IP accumulation by 38 and 34% respectively. Following vehicle pretreatment, maximal DOI-stimulated ERK activity, measured 2 min following agonist treatment, was $181 \pm 13\%$ above basal (Figure 5B). 24 h pretreatment with ketanserin, enhanced DOI-stimulated ERK activity by a maximum of $266 \pm 10\%$ over basal, suggesting a 1.8-fold reduction in constitutive desensitization. By contrast, both risperidone and paliperidone appeared to enhance constitutive desensitization (reduced DOI-stimulated ERK activity by approximately 30%), acting as agonists of comparable efficacy. DOI-stimulated ERK activity, measured at the 2 min time point, following risperidone and paliperidone pretreatment was $122 \pm 12\%$ and $126 \pm 2\%$ over basal.

Dopamine D₂ receptor signalling

Agonist activity at dopamine D₂ receptors typically results in activation of Gi family G proteins leading to an inhibition of stimulated adenylyl cyclase activity and reduction in cellular cAMP levels (Beaulieu and Gainetdinov, 2011). Consequently, dopamine D₂ inverse agonist activity is reflected by an increase in stimulated adenylyl cyclase activity and an increase in cAMP accumulation. As shown in Figure 6A, acute treatment with the reference inverse agonist, butaclamol, increased forskolin-stimulated cAMP accumulation by approximately 300%. Risperidone and paliperidone behaved as inverse agonists, enhancing forskolin-stimulated cAMP accumulation by approximately 400 and 600% respectively. Similarly, both risperidone and paliperidone behaved as inverse agonists for the dopamine D₂-ERK pathway (Figure 6B). The inverse agonist activity of risperidone was not different from that of butaclamol, reducing basal ERK activity by approximately 20%. By contrast, the inverse agonist efficacy of paliperidone exceeded that of risperidone, reducing basal ERK activity by approximately 35%. None of the ligands tested altered recruitment of β -arrestin to the dopamine D₂ receptor.

Figure 7A shows the effect of 24 h pretreatment with vehicle or dopamine D₂ ligands on concentration-response curves to the dopamine D₂ agonist, quinpirole. The maximal response and pEC₅₀ for quinpirole to inhibit forskolin-stimulated cAMP accumulation was $47 \pm 4\%$ and 10.39 ± 0.14 (0.04 nM) following 24 h pretreatment with vehicle. Prolonged treatment with an inverse agonist, by reducing constitutive dopamine D₂ receptor activity, would be expected to enhance the effect of quinpirole. By contrast, the reference ligand, butaclamol, and both risperidone and paliperidone behaved as agonists, reducing the potency and/or efficacy of quinpirole to inhibit forskolin-stimulated cAMP accumulation following 24 h pretreatment. Following 24 h pretreatment with risperidone and paliperidone, the potency of quinpirole was reduced by approximately 100-fold to 7.70 ± 0.9 (20 nM) and 7.96 ± 0.04 (11 nM) respectively. The effect of butaclamol to promote desensitization of the dopamine D₂-adenylyl cyclase system was equivalent to that of the prototypical agonist, quinpirole. Butaclamol decreased the potency and efficacy of quinpirole [pEC₅₀ = 7.55 ± 0.34 (28 nM), E_{\max} = $17 \pm 8\%$] for inhibition of adenylyl cyclase activity. Similarly, 24 h pretreatment with quinpirole decreased the maximal response of quinpirole to $13 \pm 6\%$.

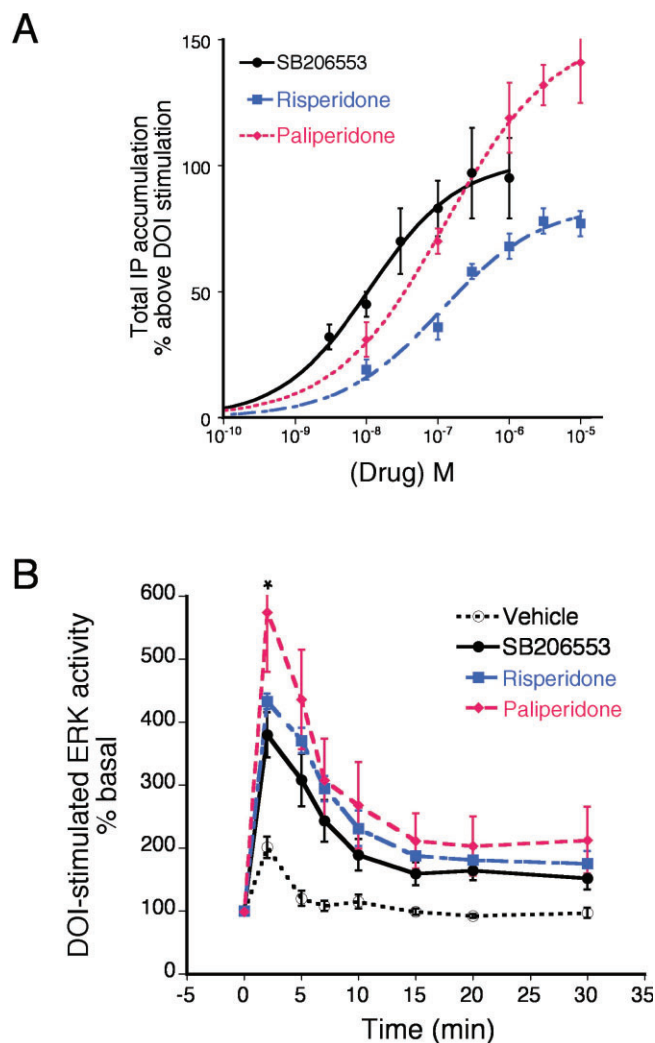


Figure 3

The effect of prolonged (24 h) pretreatment with risperidone and paliperidone compared with the reference ligand, SB 206553, on sensitization (reduction of constitutive activity) of the PLC-IP (A) and the ERK (B) responses. (A) CHO-1C19 cells, stably expressing the human 5-HT_{2C} receptor, were pretreated with the indicated concentrations of SB 206553, risperidone, paliperidone or vehicle for 24 h. Following a wash, cells were treated with a maximal concentration of the 5-HT_{2A/2C} agonist, DOI (1 μ M), for 10 min to measure IP accumulation. Data are expressed as the percent above the vehicle-pretreated effect of DOI ($94 \pm 19\%$ above basal) and represent the mean \pm SEM of three experiments. The maximal increases in DOI stimulation by SB 206553, risperidone and paliperidone were $93 \pm 5\%$, $85 \pm 7\%$ and $135 \pm 11\%$ respectively. The maximal sensitization produced by paliperidone was significantly greater than that produced by risperidone ($P < 0.05$, one-way ANOVA followed by Bonferroni's *post hoc* test). (B) CHO-1C19 cells were pretreated with maximal concentrations of SB 206553 (300 nM), risperidone (1 μ M) or paliperidone (3 μ M) for 24 h. Following a wash, cells were treated with DOI (1 μ M) for the indicated periods of time and the levels of pERK were measured. Data are expressed as the percent above basal (0 time) levels and represent the mean \pm SEM of five experiments. Pretreatment with all three ligands enhanced DOI-stimulated ERK activity. * $P < 0.05$ for the effect of paliperidone compared with that of risperidone at the 2 min time point (two-way ANOVA followed by Bonferroni's *post hoc* test).

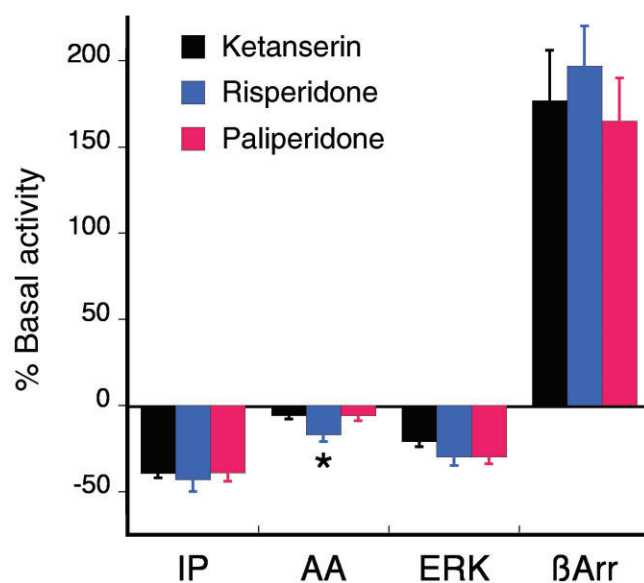


Figure 4

The effect of maximal concentrations ($100 \times K_i$) of risperidone (30 nM) and paliperidone (100 nM) compared with the reference ligand, ketanserin (1 μ M), for changes in basal signalling responses coupled to the human 5-HT_{2A} receptor. For the PLC-IP pathway, HEK-2A cells stably expressing the human 5-HT_{2A} receptor (a gift from Bryan Roth, University of North Carolina) were incubated with ligands for 30 min and IP accumulation was measured. All three ligands behaved as inverse agonists, reducing basal IP accumulation. There was no difference in the magnitude of the response between risperidone and paliperidone. For the PLA₂-AA pathway, CHO-FA4 cells were incubated with ligands for 30 min. Neither ketanserin, nor paliperidone significantly inhibited basal AA release. CHO-FA4 cells were incubated with ligands for 5 min to measure changes in ERK activity. All three ligands behaved as inverse agonists, reducing basal AA release. There was no difference in the magnitude of the response between risperidone and paliperidone. U2OS cells were treated with ligands for 90 min to measure recruitment of β -arrestin to the 5-HT_{2A} receptor. All three ligands behaved as agonists, increasing the recruitment of β -arrestin to the receptor. There was no difference in the magnitude of the effect between risperidone and paliperidone. Bars represent the mean \pm SEM of 3–6 experiments. * $P < 0.05$ for the effect of paliperidone compared with that of risperidone.

Figure 7B shows the effect of 24 h pretreatment with dopamine D₂ receptor ligands or vehicle on quinpirole-stimulated ERK activity. Following vehicle pretreatment, quinpirole increased ERK activity a maximum of $24 \pm 4\%$ above basal, measured 2 min following agonist stimulation. By contrast, butaclamol decreased quinpirole-stimulated ERK activity by about 50%, to $9 \pm 2\%$ above basal. Prolonged pretreatment with risperidone had no effect on maximal ERK stimulation by quinpirole; however, prolonged pretreatment with paliperidone produced a twofold enhancement of the maximal ERK stimulation by quinpirole.

The agonist activity of the prototypical inverse agonist, butaclamol, to act as an agonist to promote desensitization of the dopamine D₂-adenylyl cyclase and ERK pathways following 24 h pretreatment was surprising. As discussed above, prolonged treatment with inverse agonists can enhance subsequent agonist responses by reducing constitutive

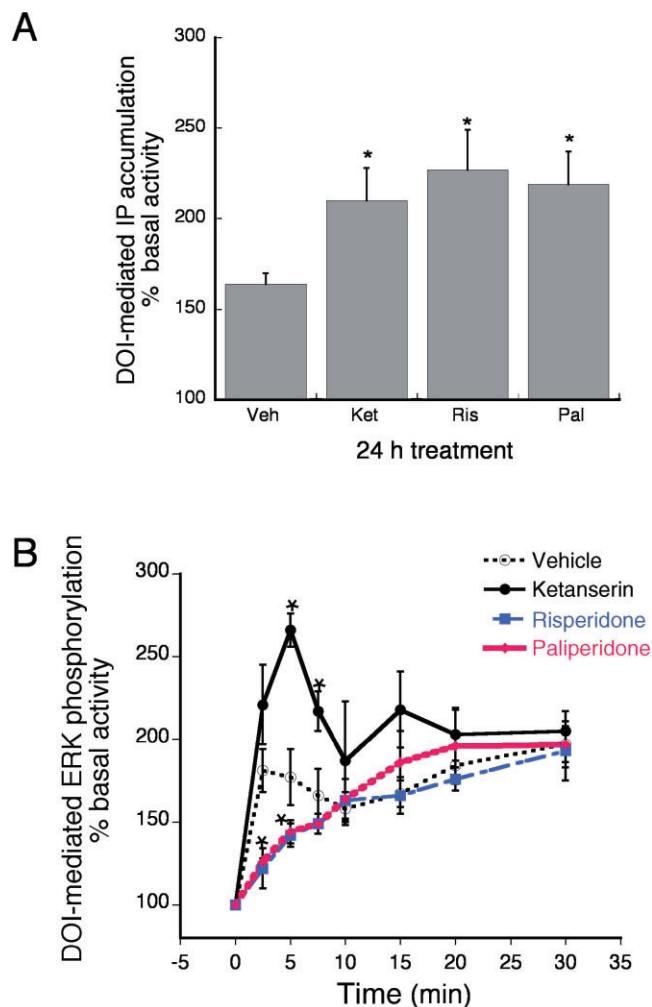


Figure 5

The effect of prolonged (24 h) pretreatment with risperidone and paliperidone compared with the reference ligand, ketanserin, on sensitization (reduction of constitutive activity) of the PLC-IP (A) and the ERK (B) responses. HEK-2A cells, stably expressing the human 5-HT_{2A} receptor, were pretreated with maximal concentrations ($100 \times K_i$) of ketanserin (1 μ M), risperidone (30 nM), paliperidone (100 nM) or vehicle for 24 h. Following a wash, cells were treated with a maximal concentration of the 5-HT_{2A/2C} agonist, DOI (1 μ M), for 10 min to measure IP accumulation (A) or ERK activity (B). (A) Data are expressed as the percent above the vehicle-pretreated effect of DOI and represent the mean \pm SEM of six experiments. All three ligands behaved as inverse agonists, enhancing the stimulation of IP accumulation produced by DOI. There was no difference in the effect of risperidone compared to that of paliperidone. $*P < 0.05$ for the ligand pretreatment effect compared to that of vehicle pretreatment. (B) Data are expressed as the percent above basal (0 time) levels and represent the mean \pm SEM of four experiments. Whereas ketanserin behaved as an inverse agonist, enhancing the effect of DOI, both risperidone and paliperidone acted as agonists, decreasing the effect of DOI. There was no difference in the effect of risperidone compared to that of paliperidone. $*P < 0.05$ for the ligand pretreatment effect compared to that of vehicle pretreatment.

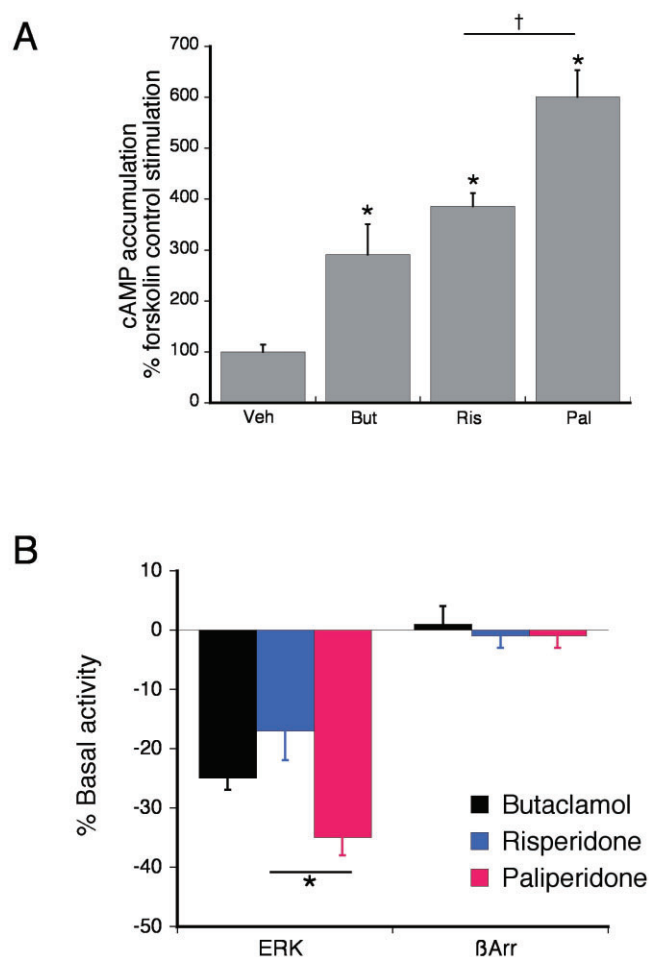
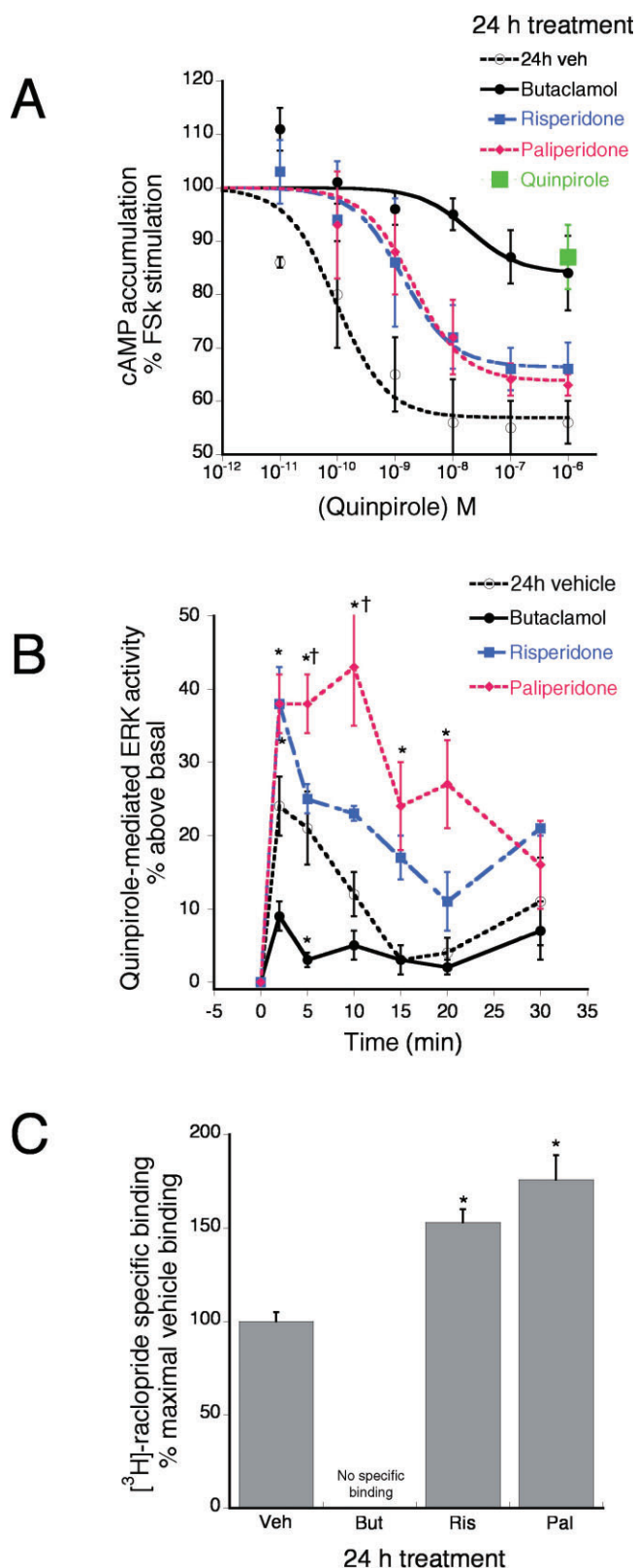


Figure 6

The effect of maximal concentrations ($100 \times K_i$) of risperidone (300 nM) and paliperidone (200 nM) compared with the reference ligand, butaclamol (1 μ M), for changes in forskolin-stimulated cAMP accumulation (A), ERK activity and recruitment of β -arrestin (B) in HEK-D2 cells stably expressing the human dopamine D₂ receptor. (A) HEK-D2 cells were incubated with ligands or vehicle for 15 min followed by incubation with the adenylyl cyclase activator, forskolin (1 μ M) for an additional 15 min. Accumulation of cAMP was measured with RIA. All three ligands behaved as inverse agonists, enhancing forskolin-stimulated cAMP accumulation. The dopamine D₂ agonist, quinpirole (1 μ M) inhibited forskolin-stimulated cAMP accumulation by $51 \pm 3\%$ (not shown). The effect of paliperidone was significantly greater than that of risperidone. Bars represent the mean \pm SEM of six experiments. $*P < 0.05$ compared to vehicle. $^\dagger P < 0.05$ for the effect of paliperidone compared with that of risperidone. (B) For ERK activity, HEK-D2 cells were incubated with ligand for 5 min. All three ligands behaved as inverse agonists, decreasing basal ERK activation. Bars represent the mean \pm SEM of five experiments $*P < 0.05$ for the effect of paliperidone compared with that of risperidone. HEK-D2 cells were incubated for 90 min with ligands to measure recruitment of β -arrestin to the dopamine D₂ receptor. None of the ligands altered β -arrestin recruitment. The agonist, quinpirole (1 μ M) increased translocation of β -arrestin by approximately 10-fold ($984 \pm 30\%$ above basal, not shown). Bars represent the mean \pm SEM of six experiments.

**Figure 7**

The effect of prolonged pretreatment with risperidone and paliperidone compared with the reference ligand, butaclamol on quinpirole-mediated inhibition of forskolin-stimulated cAMP accumulation, quinpirole-stimulated ERK activity and on dopamine D₂ receptor density. (A) CHO-D2 cells were pretreated with maximal concentrations of butaclamol (1 μ M), risperidone (300 nM), paliperidone (200 nM) or vehicle. Following a wash, cells were incubated with the indicated concentrations of quinpirole for 15 min followed by forskolin (1 μ M) for an additional 15 min. Accumulation of cAMP was measured with RIA. All three ligands acted as agonists, reducing the potency and/or efficacy of quinpirole to inhibit cAMP accumulation. There was no difference in the effect produced between risperidone and paliperidone. Data are expressed as the percent of forskolin-stimulated cAMP accumulation and represent mean \pm SEM of four experiments. (B) 24 h pretreatment with paliperidone, but not risperidone, enhanced quinpirole-stimulated ERK activity. CHO-D2 cells were pretreated with maximal concentrations of butaclamol (1 μ M), risperidone (300 nM), paliperidone (200 nM) or vehicle for 24 h. Following a wash, cells were incubated with quinpirole (1 μ M) for the indicated periods of time and pERK levels were measured. Paliperidone, acting as an inverse agonist, increased and butaclamol, acting as an agonist, decreased quinpirole stimulated ERK activity. * P < 0.05 compared to vehicle pretreatment. Data are expressed as the percent above basal (0 time) levels and represent the mean \pm SEM of four experiments. (C) The effect of prolonged treatment with maximal concentrations of butaclamol, risperidone and paliperidone on dopamine D₂ receptor density. CHO-D2 cells were treated with the ligands or vehicle as above and [³H]-raclopride binding was measured to determine maximal binding. Pretreatment with risperidone or paliperidone increased maximal [³H]-raclopride binding whereas, pretreatment with butaclamol reduced dopamine D₂ receptor density. There was no difference in the magnitude of the effect of risperidone versus paliperidone. Data are expressed as a percent of maximal binding in vehicle pretreated cells and bars represent mean \pm SEM of three experiments. * P < 0.05 compared to the vehicle pretreated condition.

desensitization. In general, two mechanisms have been reported by which prolonged treatment with inverse agonists can reduce constitutive desensitization. One mechanism involves an increase in receptor density due to decreased constitutive internalization (for a review, see Milligan and Bond, 1997). In addition, enhancement of agonist action can occur in the absence of a change in receptor density as a result of changes in receptor-effector coupling efficiency (Berg *et al.*, 1999). To examine the mechanism for butaclamol pretreatment to decrease the response to quinpirole, we measured receptor density following 24 h pretreatment with butaclamol, risperidone and paliperidone. Figure 7C shows that pretreatment with butaclamol reduced [³H]-raclopride binding from 566 ± 44 fmol-mg⁻¹ protein (vehicle pretreatment) to undetectable levels. Interestingly, 24 h pretreatment with risperidone and paliperidone increased [³H]-raclopride binding to 852 ± 117 and 1068 ± 64 fmol-mg⁻¹ protein respectively.

Histamine H₁ receptor signalling

Figure 8 shows the acute effects of treatment of cells expressing the human histamine H₁ receptor with maximal concentrations ($100 \times K_i$) of the reference ligand mepyramine, risperidone and paliperidone. For the histamine H₁-PLC

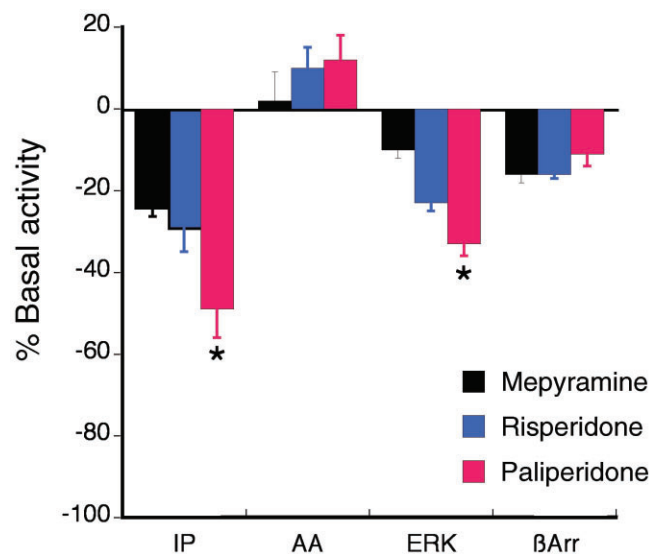


Figure 8

The effect of maximal concentrations ($100 \times K_i$) of risperidone ($2 \mu\text{M}$) and paliperidone ($2 \mu\text{M}$) compared with the reference ligand, mepyramine ($1 \mu\text{M}$), on basal IP accumulation, AA release, ERK activity and β -arrestin recruitment responses coupled to the human histamine H_1 receptor. For the PLC-IP pathway, HEK-H1 cells stably expressing the human histamine H_1 receptor were incubated with ligands for 30 min and IP accumulation was measured. All three ligands behaved as inverse agonists, reducing basal IP accumulation. The reduction in basal IP accumulation produced by paliperidone was greater than that of risperidone. For the PLA_2 -AA pathway, CHO-H1 cells were incubated with ligands for 30 min. None of the ligands significantly inhibited basal AA release. HEK-H1 cells were incubated with ligands for 5 min to measure changes in ERK activity. All three ligands behaved as inverse agonists, reducing basal AA release. The effect of paliperidone was greater than that of risperidone. CHO-H1 cells were treated with ligands for 90 min to measure recruitment of β -arrestin to the histamine H_1 receptor. All three ligands behaved as agonists, increasing the recruitment of β -arrestin to the receptor. There was no difference in the magnitude of the effect between risperidone and paliperidone. Bars represent the mean \pm SEM of 4–6 experiments. * $P < 0.05$ for the effect of paliperidone compared with that of risperidone.

pathway, all three ligands behaved as inverse agonists, reducing basal IP accumulation by $24 \pm 2\%$, $29 \pm 6\%$ and $49 \pm 7\%$, for mepyramine, risperidone and paliperidone respectively. The inverse agonist efficacy of paliperidone to reduce basal IP accumulation was significantly greater than that of risperidone. Similarly, all three ligands were inverse agonists on the histamine H_1 -ERK pathway, again with the inverse agonist efficacy of paliperidone being greater than that of risperidone. Basal ERK activity was reduced by $10 \pm 2\%$, $23 \pm 2\%$ and $33 \pm 3\%$ by mepyramine, risperidone and paliperidone respectively. For β -arrestin recruitment to the histamine H_1 receptor, mepyramine, risperidone and paliperidone were equi-efficacious inverse agonists, reducing basal β -arrestin recruitment by approximately 15%. For the histamine H_1 - PLA_2 pathway, there was no significant change in AA release by any of the three ligands tested. The agonist, histamine, produced a very weak stimulation of AA release ($20 \pm$

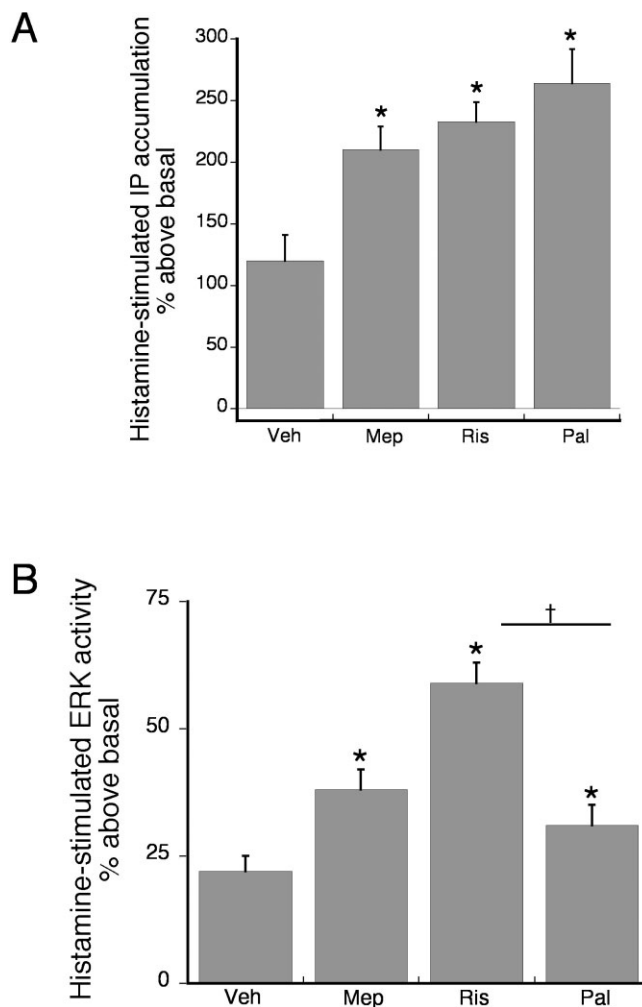


Figure 9

The effect of prolonged pretreatment with risperidone and paliperidone compared with the reference ligand mepyramine on sensitization of the PLC-IP (A) and ERK (B) pathways. Cells were treated with maximal concentrations of mepyramine ($1 \mu\text{M}$), risperidone ($2 \mu\text{M}$), paliperidone ($2 \mu\text{M}$) or vehicle for 24 h. Following a wash, histamine ($1 \mu\text{M}$)-stimulated IP accumulation or ERK activity was measured. (A) For IP accumulation, all three ligands acted as inverse agonists, enhancing the effect of histamine. There was no difference in the magnitude of the effect of paliperidone versus that of risperidone. * $P < 0.05$ compared to the vehicle pretreated condition. Bars represent the mean \pm SEM of three experiments. (B) For ERK activity, all three ligands acted as inverse agonists, enhancing histamine-stimulated ERK activity. The effect of risperidone was greater than that of paliperidone. * $P < 0.05$ compared to the vehicle pretreated condition. † $P < 0.05$ for paliperidone compared to risperidone. Bars represent the mean \pm SEM of three experiments.

4%), suggesting that the histamine H_1 receptor couples weakly to the PLA_2 signalling pathway in these cells.

Figure 9A shows the effect of 24 h pretreatment with maximal concentrations of mepyramine, risperidone and paliperidone on histamine stimulation of the histamine H_1 -PLC pathway. In cells pretreated with vehicle, histamine stimulated IP accumulation by approximately 120% (twofold) over basal. Pretreatment with mepyramine, risperidone or

paliperidone equally enhanced the histamine effect approximately twofold. Twenty-four hours pretreatment also enhanced histamine stimulation of ERK activity. Pretreatment with mepyramine and paliperidone increased histamine stimulation of ERK activity by about 55%, from $22 \pm 3\%$ above basal in vehicle pretreated cells to $38 \pm 4\%$ and $31 \pm 4\%$ respectively. The inverse agonist efficacy of risperidone was greater than that for paliperidone. Pretreatment of cells with risperidone increased histamine stimulation of ERK activity by approximately twofold, to $59 \pm 4\%$ above basal.

Discussion

Functional selectivity is a term used to describe the phenomenon whereby different ligands acting at the same receptor subtype can elicit qualitatively different effects on cell function. The underlying mechanism for functional selectivity is believed to be the formation/stabilization of unique spectra of receptor conformations that are dependent upon the structure of the ligand used. Different receptor conformations are expected to have different coupling/activation efficiencies with the various signal transduction molecules (i.e. G proteins, β -arrestins, etc.) coupled to the receptor. Thus, individual drugs have the capacity to generate a unique spectrum of activity of cellular signalling pathways. Given that some cellular pathways may lead to therapeutic effects, whereas others may mediate adverse effects, it is important to understand the functional selectivity profiles of drugs.

In a study exploring the functional selectivity of various derivatives of amphetamine at 5-HT_{2A} and 5-HT_{2C} receptor signalling to PLC and PLA₂, we found that relatively small differences in drug structure could result in significant differences in signalling. For example, 2,5-dimethoxyphenylisopropylamine (DMA) differs from 2,5-dimethoxy-4-methylphenylisopropylamine (DOM) by a single methyl group at the 4' position on the phenyl ring. At the 5-HT_{2C} receptor, DOM is a strong agonist for both PLA₂ and PLC, whereas DMA has no efficacy at PLA₂ (it is an antagonist), but retains relatively strong agonist activity at the PLC pathway.

Paliperidone is a metabolite of risperidone and differs from risperidone by only a single hydroxyl group. Although the receptor-binding characteristics are similar (Gray and Roth, 2007), in this study, we found that the signalling profiles of paliperidone acting at dopamine D₂, 5-HT_{2A}, 5-HT_{2C} and histamine H₁ receptors differed from those of risperidone. As shown in Figure 10, depending upon the receptor-response pair, the relative efficacy of paliperidone was greater than, less than or not different from that of risperidone. Such response-dependent differences in relative efficacy indicate that as a consequence of a single hydroxyl group, the receptor conformation spectrum promoted by paliperidone differs from that of risperidone. In some cases, the receptor conformations promoted by paliperidone are sufficiently different from those of risperidone that coupling to, and regulation of, cellular signalling pathways differs between the two drugs. Differential regulation of cellular signalling suggests that the physiological and behavioral consequences of paliperidone and risperidone may differ as well.

For the majority of responses measured, paliperidone and risperidone displayed either inverse agonism or antagonism. Inverse agonism, the property of a drug that decreases ligand-independent or constitutive receptor activity, is now a well-recognized pharmacological property. Preclinical studies have shown that many atypical APDs display inverse agonism at their target receptors, leading to suggestions that the inverse agonist properties of atypical APDs may be responsible for therapeutic activity (Akam and Strange, 2004; Heusler *et al.*, 2007; Meltzer *et al.*, 2012). However, in general, supportive evidence for therapeutic relevance of inverse agonism is lacking in clinical settings (for review see Parra and Bond, 2007).

Interestingly, paliperidone and risperidone were both agonists acting at the 5-HT_{2A} receptor for β -arrestin translocation and sensitization of ERK and acting at the dopamine D₂ receptor for sensitization of AC signalling. Ligands that act as agonists for some responses coupled to a receptor, but inverse agonists for other responses mediated by the same receptor [e.g. 'protean' ligands (Kenakin, 2001; Brink *et al.*, 2004)] have been identified for many GPCRs (for review, see Kenakin, 2007). The existence of protean ligands underscores the importance of specifying the response measured when referring to the pharmacological characteristics of a ligand, as the same ligand, acting at a single receptor subtype, can be both an agonist and an inverse agonist at the same time in the same cells. This also emphasizes that measurement of a single response is not sufficient to pharmacologically characterize a receptor as an agonist, antagonist or inverse agonist.

Interestingly, the dopamine D₂ reference inverse agonist ligand, butaclamol, as well as risperidone and paliperidone, behaved as agonists for sensitization of the dopamine D₂ receptor-adenylyl cyclase signalling pathway. Prolonged pretreatment resulted in reduced responsiveness to the agonist, quinpirole, to reduce adenylyl cyclase activity. In fact, the reduction in the quinpirole response produced by pretreatment with butaclamol was equivalent to that produced by pretreatment with quinpirole. Similarly, butaclamol behaved as an agonist following prolonged pretreatment when quinpirole stimulation of ERK activity was measured. Changes in receptor-effector responsiveness as a result of treatment with an agonist (desensitization) or with an inverse agonist (sensitization) can occur through changes in receptor density or changes in receptor-effector coupling efficiency as a result of changes in signal transduction components. Pretreatment with butaclamol reduced the expression of dopamine D₂ receptors, which is consistent with the reduced inhibition of adenylyl cyclase activity and reduced stimulation of ERK by quinpirole. However, pretreatment with risperidone or paliperidone produced a slight increase in dopamine D₂ receptor density. While this increase in dopamine D₂ receptor density may underlie the mechanism by which pretreatment with paliperidone enhances quinpirole-stimulated ERK activity, it does not explain why pretreatment with risperidone and paliperidone reduced quinpirole-mediated inhibition of adenylyl cyclase activity. This suggests that additional changes in the dopamine D₂ receptor-adenylyl cyclase signalling cascade may occur following risperidone and paliperidone pretreatment that results in an overall reduction in receptor-adenylyl cyclase signalling in the presence of increased receptor density.

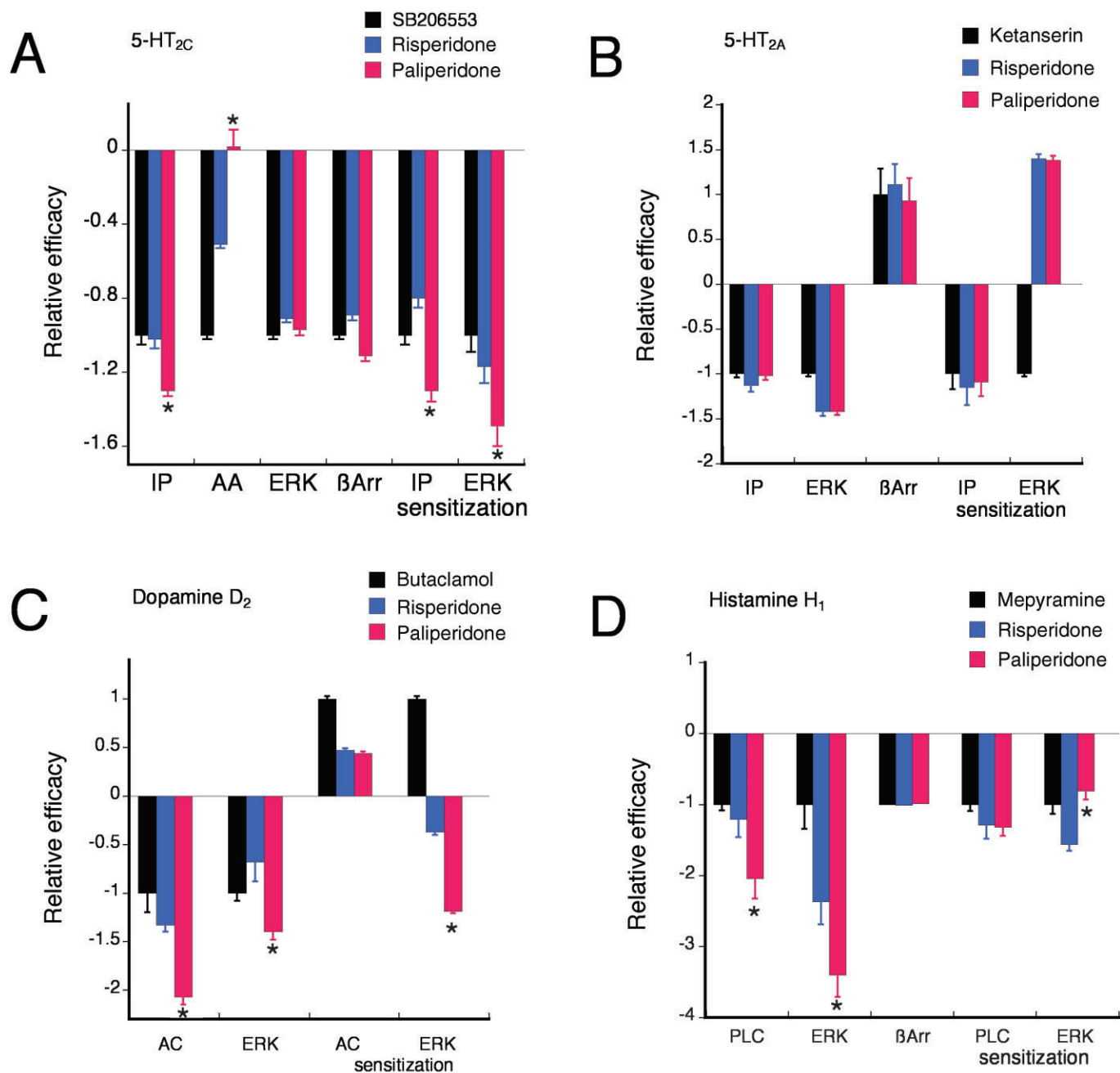


Figure 10

Relative efficacy of paliperidone versus risperidone for various cellular signalling responses coupled to human 5-HT_{2C} (A), 5-HT_{2A} (B), dopamine D₂ (C) and histamine H₁ (D) receptors. For each response, relative efficacy was calculated as the maximal response of paliperidone or risperidone divided by that of the reference ligand. Differences in relative efficacy for responses coupled to a single receptor subtype reflect functional selectivity.

Although it is difficult to extrapolate differences in the cellular actions of paliperidone versus risperidone to differences *in vivo*, Dremencov *et al.* (2007) reported recently that paliperidone differed from risperidone in the regulation of serotonergic and noradrenergic firing rates *in vivo* in rats. Unlike risperidone, which was effective at a dose of 0.4 mg·kg⁻¹ (i.v.), acute administration of paliperidone at doses up to 1 mg·kg⁻¹ did not alter the firing rate of 5-HT

neurons. Similarly, 5-HT neuronal activity was reduced following 2 day and 14 day regimens (1 mg·kg⁻¹·day⁻¹, s.c.) of risperidone, but not paliperidone, administration. Some differences were also observed in the regulation of noradrenergic neuronal firing activity following selective serotonin reuptake inhibitor (SSRI) administration suggesting that paliperidone, but not risperidone, may be effective in treating SSRI-resistant depression. Even though risperidone is

metabolized to paliperidone *in vivo*, this study suggests that the differences in cellular signalling patterns between risperidone and paliperidone observed *in vitro*, may have physiological relevance.

For several reasons, it is difficult to attribute differences in clinical effects of paliperidone versus risperidone as due to pharmacodynamic differences. Risperidone is metabolized to paliperidone and therefore paliperidone is present in patients taking risperidone. Moreover, because paliperidone is not metabolized by the liver (CYP2D6), as is risperidone, there is reduced potential for drug interactions and for the consequences of genetic polymorphisms in CYP2D6 (high vs. low metabolizers). Furthermore, the formulations of the drugs used clinically typically are different. Paliperidone is generally administered as an extended release tablet (osmotic pump), which minimizes drug plasma level fluctuations as compared to the oral, immediate release formulation of risperidone. Such different formulations have significant differences in pharmacokinetic properties. The extended release formulation of paliperidone provides relatively stable plasma concentrations as compared to the rapid changes that are characteristic of oral immediate release formulations of risperidone. As it has been suggested that the incidence of extrapyramidal symptoms may be increased by fluctuations in plasma levels of atypical antipsychotics (Ereshefsky and Mascarenas, 2003), the more stable plasma levels of paliperidone may provide for reduced extrapyramidal symptom liability and perhaps improved therapeutic efficacy.

One study has attempted a 'virtual' comparison of the effects of risperidone and paliperidone using propensity score matching of data collected from six double-blind, randomized, placebo-controlled, short-term clinical trials that involved both oral, immediate-release risperidone and oral, extended-release paliperidone (Turkoz *et al.*, 2011). When doses of paliperidone ($6\text{--}12\text{ mg}\cdot\text{day}^{-1}$) calculated to produce similar systemic drug exposure as $2\text{--}4\text{ mg}\cdot\text{day}^{-1}$ of risperidone were compared, improvement in the Positive and Negative Syndrome Scale score for paliperidone was greater than that for risperidone. Some differences were also observed in placebo-adjusted adverse events with improved tolerability of paliperidone versus risperidone. However, these results should be interpreted with caution given that comparisons were made between different formulations of the drugs with significant differences in pharmacokinetic profiles and from data collected in studies with different experimental designs.

Two recent studies have made a direct, head-to-head comparison of the effects of long-acting, injectable preparations of risperidone and paliperidone but report conflicting results (Fleischhacker *et al.*, 2011; Pandina *et al.*, 2012). In a 2011 double-blind study designed to compare paliperidone palmitate versus long-acting injectable risperidone (RIS-LAI) in patients with schizophrenia ($n = 1220$; Pandina *et al.*, 2012), paliperidone was found to be non-inferior to risperidone. Further, the tolerability and safety of paliperidone palmitate was similar to that of RIS-LAI. By contrast, a 2012 phase III, double-blind study, comparing suspensions of paliperidone palmitate and extended-release risperidone microspheres (with oral supplementation), in 749 acutely symptomatic patients (339 patients completed the study), found paliperidone to be less efficacious than risperidone (Fleischhacker *et al.*, 2011). However, measurement of plasma levels of

paliperidone and risperidone indicated that the initial dosing regimen of paliperidone in the 2012 study did not lead to a plasma level rapidly enough for therapeutic efficacy as defined as that needed to occupy $\approx 60\text{--}65\%$ of central dopamine D_2 receptors (Nyberg *et al.*, 1995; Kapur *et al.*, 2000) as did the risperidone administration procedure.

In summary, the differences in the signalling profiles of paliperidone versus risperidone observed in this study suggest that the overall cellular actions of paliperidone differ from those elicited by risperidone. It is difficult to extrapolate changes in cellular signal transduction pathways to effects on physiological functions and behaviours. Although this study has revealed differences between paliperidone and risperidone in their actions to regulate various signalling systems coupled to individual receptors, attribution of these differences to possible differences in therapeutic efficacy or in generation of adverse effects is currently not possible. Further research is needed to define which of the myriad of cellular signalling pathways coupled to a receptor is/are important in the regulation of a physiological function or behavioral response. However, from a cellular perspective, it seems clear that the effects of paliperidone differ from those of risperidone.

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Conflict of interest

None.

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