Relationship between Cerebral Sigma-1 Receptor Occupancy and Attenuation of Cocaine's Motor Stimulatory Effects in Mice by PD144418

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ABSTRACT

Psychostimulant effects of cocaine are mediated partly by agonist actions at sigma-1 (σ_1) receptors. Selective σ_1 receptor antagonists attenuate these effects and provide a potential avenue for pharmacotherapy. However, the selective and high affinity σ_1 antagonist PD144418 (1,2,3,6-tetrahydro-5-[3-(4-methylphenyl)-5-isoxazolyl]-1-propylpyridine) has been reported not to inhibit cocaine-induced hyperactivity. To address this apparent paradox, we evaluated aspects of PD144418 binding in vitro, investigated σ_1 receptor and dopamine transporter (DAT) occupancy in vivo, and re-examined effects on locomotor activity. PD144418 displayed high affinity for σ_1 sites (K_i 0.46 nM) and 3596-fold selectivity over σ_2 sites (K_i 1654 nM) in guinea pig brain membranes. No appreciable affinity was noted for serotonin

and norepinephrine transporters ($K_i > 100 \mu\text{M}$), and the DAT interaction was weak (K_i 9.0 μM). In vivo, PD144418 bound to central and peripheral σ_1 sites in mouse, with an ED_{50} of 0.22 $\mu\text{mol/kg}$ in whole brain. No DAT occupancy by PD144418 (10.0 $\mu\text{mol/kg}$) or possible metabolites were observed. At doses that did not affect basal locomotor activity, PD144418 (1, 3.16, and 10 $\mu\text{mol/kg}$) attenuated cocaine-induced hyperactivity in a dose-dependent manner in mice. There was good correlation ($r^2 = 0.88$) of hyperactivity reduction with increasing cerebral σ_1 receptor occupancy. The behavioral ED_{50} of 0.79 $\mu\text{mol/kg}$ corresponded to 80% occupancy. Significant σ_1 receptor occupancy and the ability to mitigate cocaine's motor stimulatory effects were observed for 16 hours after a single 10.0 $\mu\text{mol/kg}$ dose of PD144418.

Introduction

Selective sigma-1 (σ_1) receptor antagonists typically attenuate cocaine's locomotor stimulatory effects through mechanisms that include direct blockade of cocaine's agonist actions at the site, modulation of σ_1 receptor interactions with dopaminergic and glutamatergic signal transduction pathways that are activated by cocaine, and inhibition of the neuronal adaptations and changes in gene expression that occur as a consequence of cocaine administration (Matsumoto et al., 2003, 2014). Thus, small molecule σ_1 receptor antagonists are

under study for pharmacotherapy of psychostimulant abuse (Robson et al., 2012). Cocaine is also a σ_2 receptor agonist (Garcés-Ramírez et al., 2011), and selective σ_2 receptor antagonists can also inhibit cocaine-induced hyperactivity (Lever et al., 2014). Roles played by σ_2 receptors in cocaine's behavioral effects, however, are less well defined than those for σ_1 receptors.

Akunne and colleagues (1997) characterized PD144418 (1,2,3,6-tetrahydro-5-[3-(4-methylphenyl)-5-isoxazolyl]-1-propylpyridine; Fig. 1) as a remarkably potent and selective σ_1 receptor ligand having potential antipsychotic properties. PD144418 displayed an apparent affinity (K_i) of 0.08 nM for σ_1 sites in guinea pig brain membranes and a K_i of 1377 nM for σ_2 sites in rodent neuronal NG108-15 cell membranes. The K_i ratio indicates 17,000-fold selectivity for the σ_1 receptor subtype. Moreover, PD144418 showed weak affinities, K_i values $> 10 \mu\text{M}$, for binding to, or inhibition of, more than 45 other receptors, ion channels, and enzymes. PD144418 was classified as

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ABBREVIATIONS: ANOVA, analysis of variance; BD1063, 1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine; BMY14802, α -(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazinebutanol; DAT, dopamine transporter; DTG, 1,3-di-(*o*-tolyl)guanidine; GBR12909, 1-[2-bis-(4-fluorophenyl)methoxy]ethyl-4-(3-phenylpropyl)piperazine; ID, injected dose; [^{125}I]E-IA-DM-PE-PIPZE, *E*-N-1-(3'-[^{125}I]iodoallyl)-N'-4-(3'',4''-dimethoxyphenethyl)-piperazine; [^{125}I]RTI-121, 3 β -(4-[^{125}I]iodophenyl)tropan-2 β -carboxylic acid isopropyl ester; NET, norepinephrine transporter; PD144418, 1,2,3,6-tetrahydro-5-[3-(4-methylphenyl)-5-isoxazolyl]-1-propylpyridine; SERT, serotonin transporter; SKF10,047, *N*-allylnormetazocine.

PD144418

Fig. 1. Chemical structure of PD144418.

a σ_1 receptor antagonist based upon several assays, including the ability to attenuate mescaline-induced scratching in mice. Surprisingly, Akunne et al. (1997) reported that PD144418 did not block cocaine's motor stimulatory effects in mice. Parallel studies they conducted using BMY14802 [α -(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazinebutanol], one of the first σ receptor antagonists shown to attenuate cocaine's motor stimulatory effects (Menkel et al., 1991), also showed no effect. Akunne et al. (1997) noted that there was no clear rationale for the discrepancy.

A number of recognition sites affect cocaine stimulation of motor function (Sora et al., 2010). Therefore, the σ receptor antagonist actions of a given ligand might be masked by the involvement of other pathways. For instance, haloperidol exhibits strong dopaminergic actions in addition to antagonism of σ receptors and does not attenuate cocaine-induced locomotor hyperactivity (Witkin et al., 1993; Xu et al., 2010). PD144418, however, shows no significant in vitro affinity for dopamine, serotonin, or opioid receptors, as well as many other recognition sites. On the other hand, binding interactions of PD144418 with monoamine transporters, sites known to influence cocaine's behavioral actions (Hall et al., 2009; Sora et al., 2010), were not investigated in the initial screening assays.

There have been few subsequent studies of PD144418 despite its exceptionally high affinity and selectivity for σ_1 receptors. Gund et al. (2004) employed the PD144418 structure in molecular modeling work to help establish a σ_1 receptor pharmacophore. Navarro and coworkers (2010) used the ligand in vitro during their characterization of functional heteromers of σ_1 and dopamine D₁ receptors. They observed PD144418 blockade of cocaine-induced phosphorylation of extracellular signal-regulated kinases, a property consistent with classification of the ligand as a σ_1 receptor antagonist. No further in vivo studies of PD144418 appear to have been reported since the original work of Akunne and colleagues (1997).

In the present study, we confirmed the high σ_1 receptor subtype affinity and selectivity previously reported for PD144418 and added to the in vitro binding profile by assessing interactions of PD144418 with the dopamine transporter (DAT), as well as with serotonin (SERT) and norepinephrine (NET) transporters. Furthermore, we investigated the occupancy of central and peripheral σ_1 receptors by PD144418 as a function of dose and time in normal mice using in vivo radioligand binding techniques. We then evaluated the effects of PD144418 on basal locomotor activity and cocaine-induced locomotor hyperactivity in mice. Antagonism of cocaine's stimulatory effects was indeed observed. Having both behavioral and cerebral occupancy measures provided an opportunity to examine correlation of antagonist occupancy of σ_1 receptors with attenuation of cocaine-induced locomotor hyperactivity, a relationship not previously addressed.

Materials and Methods

Drugs and Chemicals. PD144418 oxalate, BD1063 dihydrochloride (1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine), GBR12909 dihydrochloride (1-[2-[bis-(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine), desipramine hydrochloride, and fluoxetine hydrochloride were obtained from Tocris Bioscience (Minneapolis, MN). (–)-Cocaine hydrochloride, haloperidol, DTG [1,3-di-(*o*-tolyl)guanidine], and (+)-pentazocine were purchased from Sigma-Aldrich (St. Louis, MO). [¹²⁵I]E-IA-DM-PE-PIPZE [E-N-1-(3'-iodoallyl)-N'-4-(3'',4''-dimethoxyphenethyl)-piperazine; Lever et al., 2012] and [¹²⁵I]RTI-121 [3β-(4-iodophenyl) tropan-2β-carboxylic acid isopropyl ester; Lever et al., 1996] were prepared as previously described. Tritiated radioligands were obtained from PerkinElmer Life Sciences, Inc. (Boston, MA). Other chemicals and solvents were the best grades commercially available and were used as received. Drug concentrations are given in molar units. Mass amounts designated refer to the salt or free base forms listed above.

Animals. Male CD-1 mice, typically 23–28 g (Charles River Laboratories International, Inc., Wilmington, MA), were group housed in temperature- and humidity-controlled quarters under a 12-hour light/dark cycle with free access to standard rodent chow and water. Experiments were conducted during the light phase of the cycle after animals had acclimated for at least 1 week. Studies were performed in compliance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health and with prior approvals from the Institutional Animal Care and Use Committees of the University of Missouri and the Harry S. Truman Memorial Veterans' Hospital.

In Vitro Binding Studies. Stock solutions of PD144418 oxalate were prepared in dimethylsulfoxide at ambient temperature, with serial dilutions made using pH 7.4 Tris-HCl buffer (50 mM) containing 0.01% bovine serum albumin. Final assay concentrations of dimethylsulfoxide were kept at or below 1%. Stock and serial dilutions of haloperidol were prepared in distilled water containing 1% ethanol and 0.1% acetic acid. PD144418 stocks and serial dilutions were also prepared in this aqueous vehicle, but vigorous heating was required, suggesting potential issues with precipitation or adsorption to glass surfaces. Radioligands as well as brain membranes were formulated in the Tris-HCl buffers (50 mM) appropriate for each experiment. Assays were terminated by addition of ice-cold buffer followed by manifold filtration through glass fiber filters (GF/B) that had been pretreated with polyethyleneimine (0.5%). Tubes and filter paper discs were washed (3 × 5 ml) with cold buffer, and the discs were dried under vacuum. ¹²⁵I radioactivity was measured with 78% efficiency using an automated gamma counter (Wallac Wizard, Model 1480; Turku, Finland). Tritium was extracted from filter discs by standing for ≥24 hours in cocktail (OptiPhase HiSafe 2; PerkinElmer Life Sciences, Inc.) and then measured at 44% efficiency by liquid scintillation counting (Wallac Model 1409).

Binding assays for σ receptors were performed using 1.0 nM [³H](+)-pentazocine (σ_1), 3.0 nM [³H]DTG/500 nM (+)-pentazocine (σ_2), and membranes from fresh-frozen, male Hartley guinea pig brains (Rockland Immunochemicals, Gilbertsville, PA) as previously described (Lever et al., 2006). Nonspecific binding was defined by haloperidol (1.0 μ M; σ_1) or by DTG (100 μ M; σ_2). Additional σ_1 receptor assays used [¹²⁵I]E-IA-DM-PE-PIPZE at 1.0 nM, with haloperidol (1.0 μ M) to define nonspecific binding, in membranes prepared from whole CD-1 mouse brains, harvested after euthanasia by cervical dislocation, as previously described (Lever et al., 2012). Binding assays for monoamine transporters were performed, using established procedures, in membranes prepared from fresh CD-1 mouse brains. In brief, DAT assays used [¹²⁵I]RTI-121 (15 pM) in striatal membranes with GBR12909 (0.5 μ M) to define nonspecific binding (Boja et al., 1995). NET assays used [³H]nisoxetine (0.4 nM) in cortical membranes with desipramine (1.0 μ M) to define nonspecific binding (Tejani-Butt et al., 1990). SERT assays used [³H]paroxetine (0.3 nM) in whole brain membranes with fluoxetine (10 μ M) to define nonspecific binding (Hirano et al., 2005).

For the six types of binding assays performed, specific radioligand binding was 77–92% of the total binding. Experiments were conducted in duplicate or in triplicate and replicated three to six times. Data were analyzed by nonlinear regression using a sigmoidal four-parameter logistic fit (Prism 6.0c; GraphPad Software, Inc., La Jolla, CA). K_i values were calculated from IC₅₀ data by the Cheng-Prusoff (Cheng and Prusoff, 1973) relationship. Input K_d values used were 2.3 nM for [³H](+)-pentazocine and 23.9 nM for [³H]DTG (Lever et al., 2006), 3.79 nM for [¹²⁵I]E-IA-DM-PE-PIPZE (Lever et al., 2012), and 0.12 nM for [¹²⁵I]RTI-121 (Boja et al., 1995).

In Vivo Binding Studies. Dose-response data for inhibition of [¹²⁵I]E-IA-DM-PE-PIPZE binding to σ_1 receptors by PD144418 was determined in nonfasted mice. Stock solutions of PD144418 oxalate in sterile bacteriostatic saline (0.9% NaCl, 0.9% benzyl alcohol; w/v) were prepared with gentle heat and vortex mixing. Groups of animals ($n = 4$) received six doses of PD144418 oxalate (0.01–10.0 μ mol/kg), equally spaced on log scale, in sterile 0.9% saline (0.1 ml) by intraperitoneal injection 1 minute before intravenous administration of [¹²⁵I]E-IA-DM-PE-PIPZE (2.5 μ Ci) in sterile saline (0.1 ml) containing 2% ethanol through a lateral tail vein. To assess the duration of occupancy, additional groups of mice ($n = 3$ –4) received PD144418 (10.0 μ mol/kg i.p., saline) 4, 16, and 24 hours before intravenous administration of radioligand. A PD144418 dosage of 10 μ mol/kg was selected for the duration studies based upon the high occupancy of σ_1 receptors observed at this level in the dose-response experiments. Control animals ($n = 8$) received saline vehicle (0.1 ml) by intraperitoneal injection 1 minute before intravenous administration of radioligand. An additional treatment group ($n = 8$) was treated with BD1063 (5.0 μ mol/kg) in saline (0.1 ml) by intravenous injection 5 minutes before the radioligand to define nonspecific binding as previously established (Lever et al., 2012). All groups of animals were euthanized by cervical dislocation 30 minutes after radioligand administration. Whole brain, heart, lung, and spleen were harvested and weighed. Tissue samples, along with standard dilutions of the injected dose (ID), were counted for ¹²⁵I radioactivity at 78% efficiency, and the percentage ID per gram wet weight of tissue was calculated.

Studies of the effects of PD144418 on the regional cerebral distribution of [¹²⁵I]E-IA-DM-PE-PIPZE binding to σ_1 receptors were conducted similarly. Groups of mice ($n = 4$) received either saline vehicle (0.1 ml i.p.) or PD144418 (1.0 μ mol/kg i.p., saline) 1 minute before intravenous administration of [¹²⁵I]E-IA-DM-PE-PIPZE (2.5 μ Ci). Nonspecific binding was defined by a group ($n = 4$) that received BD1063 (5.0 μ mol/kg) in saline (0.1 ml) by intravenous injection 5 minutes before the radioligand. Animals were euthanized by cervical dislocation 30 minutes after radioligand administration. Whole brain was removed, and samples of prefrontal cortex, other cortex (temporal/parietal), olfactory tubercles, striatum, hypothalamus, thalamus, inferior/superior colliculi, pons/medulla, and cerebellum were dissected on an ice-cold glass plate. These tissues, along with the remainder of the brain, were lightly blotted, weighed, and counted for radioactivity along with standard dilutions of the injected dose as described above.

Ligand effects on DAT specific binding were assessed by intraperitoneal treatment of groups of mice ($n = 4$) with either PD144418 (10.0 μ mol/kg) in saline (0.1 ml) or saline vehicle (0.1 ml) 1 minute before intravenous administration of [¹²⁵I]RTI-121 (2.5 μ Ci) in saline (0.1 ml) containing 2% ethanol. Animals were euthanized by cervical dislocation 30 minutes after radioligand administration, whole brain removed, and samples of cerebellum, striatum and olfactory tubercles were dissected, weighed, and counted along with standards of the injected dose as described above. Radioactivity levels in cerebellum served to define nonspecific binding (Lever et al., 1996; Desai et al., 2005).

Two-tailed, unpaired t tests at the 95% confidence level or analysis of variance (ANOVA) ($\alpha = 0.05$) with post hoc Dunnett's or Tukey's tests (Prism 6.0c) were employed to analyze potential differences between groups. Dose-response data were fit using an unconstrained sigmoidal regression algorithm, and the regional brain uptake of [¹²⁵I]E-IA-DM-PE-PIPZE was investigated using Pearson's product-moment correlation (Prism 6.0c).

Locomotor Activity Studies. Effects of PD144418 on basal locomotor activity and cocaine-induced locomotor stimulation in mice were evaluated by procedures similar to those used previously for evaluations of other σ receptor ligands (Rodvelt et al., 2011; Sage et al., 2013). Experiments were performed in activity monitors (Model ENV-515; Med Associates Inc., Georgia, VT) consisting of a transparent box surrounded by banks of infrared sensors that were connected to a computer. Behavior was measured automatically by Med Associates' Activity Monitor (v. 4.31) software. Groups of mice ($n = 7$ –12) were acclimated to the monitors for 30–60 minutes on 2 consecutive days. On the third consecutive day, mice were placed into monitors for 45 minutes, injected intraperitoneally (5 ml solution/kg body weight) with 0.1, 1.0, 3.16, 10.0, or 31.6 μ mol/kg PD144418 or sterile 0.9% saline vehicle, returned to the monitor for 15 minutes, injected intraperitoneally with either cocaine (66 μ mol/kg, 20 mg/kg) or saline vehicle, and returned to the monitor for 60 minutes. Additional groups of mice ($n = 10$) received intraperitoneal PD144418 (10.0 μ mol/kg) 4, 16, and 24 hours before the administration of cocaine as described above. In a follow-up experiment, groups of mice ($n = 9$ –11) also were administered intraperitoneal PD144418 (3.16 μ mol/kg) or saline followed 15 minutes later by a larger dose of intraperitoneal cocaine (99 μ mol/kg, 30 mg/kg) as described above.

Distance traveled (centimeters) during the 60-minute period after cocaine or saline injections was analyzed by three-way repeated-measures ANOVA performed with PD144418 dose (0, 0.1, 1, 3.16, 10.0, and 31.6 μ mol/kg) and cocaine dose (0 and 66 μ mol/kg) as between-group factors and time (12 5-minute intervals) as a within-subjects factor. Where appropriate, $P < 0.05$, simple main effect, and Tukey's post hoc analyses were performed. To evaluate the dose-response relationship, total distance traveled during the 60-minute period after cocaine injection was summed for each group of mice, and data were analyzed by nonlinear regression using an unconstrained sigmoidal fit (Prism 6.0c). For the follow-up experiment, a three-way repeated-measures ANOVA was performed with PD144418 dose (0 and 3.16 μ mol/kg) and cocaine dose (0 and 99 μ mol/kg) as between-group factors and time as a within-subject factor.

Results

In Vitro Binding Studies. Binding affinities of PD144418 for the σ receptor subtypes determined in the present study, along with those of Akunne et al. (1997), are given in Table 1. Data from side-by-side determinations for haloperidol in the two studies are included for comparison. We observed a σ_1 receptor K_i of 0.46 ± 0.04 nM for PD144418 against [³H](+)-pentazocine in guinea pig brain membranes. PD144418 displayed a higher apparent affinity, $K_i = 0.19 \pm 0.05$ nM, for σ_1 receptors when tested against the selective σ_1 radioligand [¹²⁵I]E-IA-DM-PE-PIPZE in membranes from whole CD-1 mouse brain. These values were somewhat higher than the σ_1 receptor K_i of 0.08 nM previously reported for PD144418. The K_i determinations for haloperidol were uniformly 1.2 nM between the studies and across tissue type and radioligand. The σ_2 receptor K_i of 1654 ± 86 nM found for PD144418 in guinea pig brain membranes, using [³H]DTG in the presence of 500 nM (+)-pentazocine to mask σ_1 sites, was a close match to the K_i of 1377 nM found by Akunne et al. (1997) using [³H]DTG/(+)-pentazocine in NG108-15 cell membranes.

The affinity of PD144418 for monoamine transporters was tested using established binding assays in membranes prepared from mouse brain (Table 1). A weak interaction, $K_i = 9038$ nM, was determined for PD144418 binding to striatal DAT that was accompanied by a steep Hill slope (n_H 1.60 ± 0.06). No appreciable affinity was observed for NET and SERT,

TABLE 1

Apparent affinities, K_i values (nM), from in vitro binding studies of PD144418 and haloperidol
Means $\pm$ S.E.M., $n = 3-6$.

Receptor and Transporter Affinities			
Sigma receptors			
PD144418			
σ_1 0.46 ± 0.04 ; n_H 0.83 ± 0.07	σ_2 1654 ± 86 ; n_H 1.12 ± 0.23	σ_2/σ_1 3596^a	
σ_1 0.19 ± 0.05 ; n_H 0.81 ± 0.05^b			
σ_1 0.08 ± 0.01	σ_2 1377 ± 179	σ_2/σ_1 $17,212^c$	
Haloperidol			
σ_1 1.21 ± 0.10 ; n_H 1.08 ± 0.03	σ_2 18.1 ± 1.4 ; n_H 0.92 ± 0.06	σ_2/σ_1 15^a	
σ_1 1.24 ± 0.04 ; n_H 0.74 ± 0.05^b			
σ_1 1.2 ± 0.20	σ_2 26.0 ± 5.4	σ_2/σ_1 22^c	
Monoamine transporters			
PD144418			
DAT ^d 9038 ± 1345 ; n_H 1.60 ± 0.06			
NET ^e $> 100,000$			
SERT ^f $> 100,000$			

^a σ_1 [³H](+)-pentazocine, guinea pig brain membranes; σ_2 [³H]DTG/500 nM (+)-pentazocine, guinea pig brain membranes.

^b[¹²⁵I]E-IA-DM-PE-PIPZE, mouse brain membranes.

^cAkunne et al., 1997: σ_1 [³H](+)-pentazocine, guinea pig brain membranes; σ_2 [³H]DTG/200 nM (+)-pentazocine, NG108-15 cell membranes.

^d[¹²⁵I]RTI-121, mouse brain striatal membranes.

^e[³H]Nisoxetine, mouse brain cortical membranes; $\leq 15\%$ inhibition at 100,000 nM.

^f[³H]Paroxetine, whole mouse brain membranes; $\leq 5\%$ inhibition at 100,000 nM.

with K_i values $>100,000$ nM using cortical and whole brain membranes, respectively.

In Vivo Binding Studies. To define the interactions of PD144418 with σ_1 receptors in vivo, a series of binding studies were performed in male CD-1 mice using the selective σ_1 receptor radioligand [¹²⁵I]E-IA-DM-PE-PIPZE (Lever et al., 2012, 2014). The effects of pretreatments with PD144418 and the σ_1 receptor antagonist BD1063 on radioligand uptake in mouse whole brain and peripheral organs are shown in Fig. 2A. Animals were euthanized, and tissue radioactivity was determined 30 minutes after intravenous administration of [¹²⁵I]E-IA-DM-PE-PIPZE. As expected from previous work (Lever et al., 2012, 2014), significant blockade of uptake by BD1063 (5.0 $\mu\text{mol/kg}$ i.v.) in brain (84%), heart (58%), spleen (70%), and lung (68%) was observed. PD144418 administered intraperitoneally at 1.0 $\mu\text{mol/kg}$ proved nearly as effective at blocking radioligand binding to σ_1 receptors as BD1063 administered intravenously at 5.0 $\mu\text{mol/kg}$. In the presence of PD144418, levels of tissue radioactivity were significantly reduced by 69% in brain, 63% in heart, 48% in spleen, and 63% in lung (ANOVA, Dunnett's).

The regional distribution of [¹²⁵I]E-IA-DM-PE-PIPZE in mouse brain, which has not been previously reported, is shown in Fig. 2B. The highest levels of radioligand uptake were in cerebellum, hypothalamus, and the brain stem (pons/medulla, superior/inferior colliculi). Intermediate levels were observed for the cortical regions, whereas the lowest levels were noted for striatum, olfactory tubercles, and hippocampus. Both PD144418 (1.0 $\mu\text{mol/kg}$ i.p.) and BD1063 (5.0 $\mu\text{mol/kg}$ i.v.) significantly inhibited radioligand uptake in all regions with respect to saline controls (ANOVA, Dunnett's). The BD1063 dosage blocked uptake by 82–89% across the regions and can be used to define nonspecific radioligand binding throughout the brain. The PD144418 dosage blocked uptake by 52–67% across all regions. There were no significant differences between radioactivity levels in the various regions in the presence of BD1063, except for a small differential between the olfactory tubercles compared with the pons/medulla (ANOVA, Tukey's).

The pattern of distribution shown in Fig. 2B indicates that [¹²⁵I]E-IA-DM-PE-PIPZE uptake reflects the regional densities

of σ_1 receptors in the brain that are known from previous studies. As shown in Fig. 2C, good Pearson correlation ($r^2 = 0.78$, $P = 0.01$) was observed between specific radioligand binding, as percentage ID per gram, in seven brain regions that could be reasonably matched to specific binding data, as femtomoles per milligram tissue, from an ex vivo autoradiography study of [³H]SKF10,047 (*N*-allylnormetazocine) binding to σ_1 receptors at 60 minutes in CD-1 mouse brain (Bouchard et al., 1996). Specific [¹²⁵I]E-IA-DM-PE-PIPZE binding was obtained by subtracting the corresponding regional values for nonspecific binding as defined by BD1063. The correlation also held when total binding uptake values were used, because the levels of nonspecific binding are low in all regions. Furthermore, the residual specific binding of [¹²⁵I]E-IA-DM-PE-PIPZE after administration of PD144418 (1.0 $\mu\text{mol/kg}$ i.p.; Fig. 2B) also correlated with the ex vivo autoradiographic data ($r^2 = 0.78$, $P = 0.01$; data not shown). This indicates that PD144418 inhibits radioligand binding to cerebral σ_1 receptors in proportion to the relative site densities of the brain regions.

Dose-response studies were then performed using intraperitoneal PD144418 pretreatments at six dosage levels, equally spaced on log scale, that ranged from 0.01 to 10.0 $\mu\text{mol/kg}$ (Fig. 3). Specific binding of [¹²⁵I]E-IA-DM-PE-PIPZE to σ_1 receptors in each tissue sample was determined by subtracting the nonspecific binding for that tissue as defined by values from mice pretreated with BD1063 at 5 $\mu\text{mol/kg}$ (i.v.). PD144418 inhibition of σ_1 receptor binding proved dose-dependent in all tissues examined. Data were fit ($r^2 = 0.99$) to sigmoidal curves to calculate the effective dose for 50% occupancy (ED_{50}). PD144418 exhibited an ED_{50} of 0.22 $\mu\text{mol/kg}$ in whole brain and also was potent inhibitor of σ_1 receptor binding in the heart (ED_{50} 0.08 $\mu\text{mol/kg}$) and the lung (ED_{50} 0.11 $\mu\text{mol/kg}$). The ligand proved somewhat less effective in spleen, with an ED_{50} of 0.39 $\mu\text{mol/kg}$.

Although PD144418 showed only weak affinity for the DAT in vitro (Table 1), the possible in vivo occupancy of this site by unknown metabolites was tested using the radioligand [¹²⁵I]RTI-121. The specific binding of [¹²⁵I]RTI-121 to DAT in striatum and olfactory tubercles was assessed as the difference

Fig. 2. Biodistribution of σ_1 -receptor radioligand [^{125}I]E-IA-DM-PE-PIPZE in mouse brain and peripheral organs at 30 minutes and effects of PD144418 and BD1063 pretreatments. (A) Radioligand uptake is significantly inhibited (ANOVA, Dunnett's) in whole brain and peripheral organs by both PD144418 (1.0 $\mu\text{mol/kg}$ i.p.) and BD1063 (5.0 $\mu\text{mol/kg}$ i.v.). Data represent mean $\pm$ S.E.M., $n = 4$ –8 per group. (B) Radioligand uptake in mouse brain regions is fully blocked by BD1063 (5.0 $\mu\text{mol/kg}$ i.v.) and partially inhibited by PD144418 (1.0 $\mu\text{mol/kg}$ i.p.) in all regions examined (ANOVA, Dunnett's). Data represent mean $\pm$ S.E.M., $n = 4$ per group. (C) Specific [^{125}I]E-IA-DM-PE-PIPZE binding in mouse brain regions correlates (Pearson $r^2 = 0.78$,

in percentage ID per gram between these regions that are rich in the DAT and that in cerebellum, a brain region with a low density of the sites that can be used as an internal reference for nonspecific binding (Lever et al., 1996; Desai et al., 2005). Mice pretreated with PD144418 at 10.0 $\mu\text{mol/kg}$ (i.p.) showed no difference (t test, $P > 0.05$; data not shown) from saline controls in the levels of specific [^{125}I]RTI-121 binding observed for the striatum (3.66 ± 0.37 versus 3.96 ± 0.17 %ID/g) or for the olfactory tubercles (1.95 ± 0.25 versus 1.96 ± 0.23 %ID/g).

Locomotor Activity Studies. The effects of PD144418 on basal locomotor activity and cocaine-induced locomotor stimulation in male CD-1 mice are shown in Fig. 4. After habituation, groups of animals were treated with PD144418 (0.10–31.6 $\mu\text{mol/kg}$ i.p.) or saline followed 15 minutes later by either cocaine (20 mg/kg or 66 $\mu\text{mol/kg}$ i.p.) or saline. Figure 4A presents total distance traveled for the 60-minute period after the cocaine or saline injections, whereas Fig. 4B depicts the full time course of each dose. A significant main effect of cocaine dose was found [$F_{(1,91)} = 46.858$, $P < 0.001$] as expected, and mice administered 66 $\mu\text{mol/kg}$ cocaine displayed nearly sixfold higher locomotor activity than mice administered only saline. Significant interactions of PD144418 dose $\times$ cocaine dose [$F_{(5,91)} = 5.235$, $P < 0.001$] and PD144418 dose $\times$ cocaine dose $\times$ time [$F_{(55,1001)} = 1.631$, $P = 0.026$] also were observed. Post hoc Tukey's analyses revealed that mice administered 31.6 $\mu\text{mol/kg}$ (11.8 mg/kg) PD-144418 followed by saline were 70% less active than animals that received only saline. There were no significant differences in locomotor activity among the groups of mice that received 0.10, 1.0, 3.16, or 10.0 $\mu\text{mol/kg}$ PD144418 followed by saline and the control group that received only saline injections.

PD144418 treatments produced a dose-dependent attenuation of the locomotor stimulation induced by 66 $\mu\text{mol/kg}$ cocaine (Fig. 4). Analysis of total distance traveled revealed that mice administered 1.0, 3.16, 10.0, or 31.6 $\mu\text{mol/kg}$ PD144418 followed by cocaine were 40–85% less active than mice administered saline followed by 66 $\mu\text{mol/kg}$ cocaine (Fig. 4A). Furthermore, mice administered 31.6 $\mu\text{mol/kg}$ PD144418 and cocaine were less active than mice administered 1.0 $\mu\text{mol/kg}$ PD144418 and cocaine. There was not a significant difference in locomotor activity between the group of mice that received 0.10 $\mu\text{mol/kg}$ PD144418 followed by cocaine and those that received saline followed by cocaine.

Regarding the time course (Fig. 4B), there was less activity for mice administered 31.6 $\mu\text{mol/kg}$ PD144418 followed by 66 $\mu\text{mol/kg}$ cocaine than for mice administered saline followed by cocaine at the 20- to 75-minute time points. Mice administered 10 $\mu\text{mol/kg}$ PD144418 and cocaine were less active than mice administered saline and cocaine at the 20- to 45-minute time points. Mice administered 3.16 $\mu\text{mol/kg}$ PD144418 and cocaine were less active than mice administered saline and cocaine at the 20- to 45-minute time points. There was less activity for mice administered 1.0 $\mu\text{mol/kg}$ PD144418 followed by cocaine compared with mice administered saline followed by cocaine at the 20- to 35-minute time points. However, there were no significant differences in locomotor activity at any time point between the groups of mice that received 0.10 $\mu\text{mol/kg}$

$P = 0.01$) with relative σ_1 receptor densities known from prior autoradiographic studies (Bouchard et al., 1996). Biodistribution data are mean values, $n = 4$ per group (see B).

Fig. 3. PD144418 (0.01–10.0 $\mu\text{mol/kg}$ i.p.) dose dependently inhibits specific [^{125}I]E-IA-DM-PE-PIPZE binding to σ_1 receptors at 30 minutes in whole mouse brain (A), heart (B), spleen (C), and lung (D). Data represent mean $\pm$ S.E.M., $n = 4$ –8 per group. Nonspecific radioligand binding was defined by a separate group of animals ($n = 8$) that received BD1063 (5.0 $\mu\text{mol/kg}$ i.v.). Curves represent the sigmoidal logistic fits ($r^2 = 0.99$, A–D) used to obtain ED_{50} values.

PD144418 followed by cocaine and those that received saline followed by cocaine.

In a follow-up study, the effect of PD144418 (3.16 $\mu\text{mol/kg}$) on locomotor stimulation induced by 99 $\mu\text{mol/kg}$ cocaine was assessed. Total distance traveled for the 60-minute period after cocaine injection is presented in Fig. 4A. A significant main effect of cocaine dose [$F_{(1,45)} = 48.720$, $P < 0.001$] was found, because mice-administered cocaine displayed greater activity than mice-administered saline. A significant PD144418 dose $\times$ cocaine dose also was observed [$F_{(1,35)} = 4.549$, $P = 0.004$], although the PD144418 dose $\times$ cocaine dose $\times$ time interaction was not significant [$F_{(11,385)} = 0.821$, $P = 0.621$]. Mice-administered 3.16 $\mu\text{mol/kg}$ PD144418 followed by cocaine were less active than mice-administered saline followed by cocaine. Thus, PD144418 decreased the locomotor stimulation induced by the lower (66 $\mu\text{mol/kg}$) and the higher (99 $\mu\text{mol/kg}$) cocaine doses by 57 and 31%, respectively.

Correlation of σ_1 Receptor Occupancy with Attenuation of Cocaine's Motor Stimulatory Effects. An inverse relationship between PD144418 binding to cerebral σ_1 receptors in vivo and levels of cocaine-induced locomotor stimulation can be seen by inspection of Figs. 3A and 4A. Explicit comparison of those data, as percentage of control values, is presented in Fig. 5A. Reductions in radioligand specific binding by PD144418 have been transformed to σ_1 receptor occupancies, and total distance traveled after 66 $\mu\text{mol/kg}$ cocaine administration has been transformed, by subtraction of average basal activity, to represent hyperactivity. A behavioral ED_{50} of 0.79 $\mu\text{mol/kg}$ for PD144418

attenuation of cocaine-induced locomotor stimulation was obtained from the sigmoidal curve of Fig. 5A, although the fit was not robust ($r^2 = 0.47$). As noted earlier, PD144418 exhibited an ED_{50} of 0.22 $\mu\text{mol/kg}$ for occupancy of σ_1 sites in whole brain (Fig. 3A). A significant, one-tailed Pearson correlation ($r^2 = 0.88$, $P = 0.01$; Fig. 5B) of σ_1 receptor occupancy with attenuation of cocaine-induced locomotor hyperactivity was observed for the five (X,Y) data pairs from these experiments. Two data clusters that represent extremes in occupancy and observed effects define this linear relationship. One corresponds to sets of animals showing a 50 to 70% reduction in hyperactivity that is associated with 80 to 95% σ_1 receptor occupancy by PD144418 (1–10 $\mu\text{mol/kg}$). The other corresponds to the saline-treated controls, and a set of animals receiving the lowest dose of PD144418 (0.1 $\mu\text{mol/kg}$) where no effect on hyperactivity was observed at 20% σ_1 receptor occupancy. Thus, approximately 80% σ_1 receptor occupancy by PD144418 leads to a 50% reduction in the motor stimulatory effects of cocaine.

To investigate relationships between the duration of PD144418 occupancy of σ_1 receptors and the attenuation of cocaine-induced locomotor hyperactivity, we performed supplemental 4-, 16-, and 24-hour studies using a 10.0 $\mu\text{mol/kg}$ dosage. Figure 6 shows the effects of PD144418 pretreatments on the binding of [^{125}I]E-IA-DM-PE-PIPZE to σ_1 receptors in mouse whole brain and peripheral organs as a function of time. Significant inhibition of specific radioligand binding was observed through the 16-hour pretreatment period for all tissues examined, whereas data from the 24-hour pretreatment did not

Fig. 4. PD144418 attenuates cocaine-induced hyperactivity in mice. Animals were placed in an activity monitor for 45 minutes, injected (intraperitoneally) with PD144418 (0.10–31.6 $\mu\text{mol/kg}$) or saline, returned to the monitor for 15 minutes, injected (intraperitoneally) with cocaine (66 or 99 $\mu\text{mol/kg}$) or saline, and then monitored for 60 minutes. (A) Distance traveled (centimeters) after the second saline or cocaine injection, summed across the 60-minute postinjection period. Significant differences (ANOVA, Tukey's): *groups given PD144418 and saline that differ from the group given only saline injections; *groups administered PD144418 and cocaine (66 $\mu\text{mol/kg}$) that differ from the group given saline followed by 66 $\mu\text{mol/kg}$ cocaine; **group administered PD144418 (31.6 $\mu\text{mol/kg}$) and cocaine (66 $\mu\text{mol/kg}$) that differs from the group given PD144418 (1.0 $\mu\text{mol/kg}$) and cocaine (66 $\mu\text{mol/kg}$); and # group given PD144418 and cocaine (99 $\mu\text{mol/kg}$) that differs from the group given saline followed by 99 $\mu\text{mol/kg}$ cocaine. Data represent mean $\pm$ S.E.M., $n = 7$ –13 per group. (B) Distance traveled (centimeters) in 5-minute intervals. Arrows designate PD144418 or saline injections (time = 0) and 66 $\mu\text{mol/kg}$ cocaine or saline injections (time = 15). Closed symbols represent mice administered cocaine, and open symbols represent mice administered saline. Significant differences at various times between the PD144418 treatment groups, with respect to the group given saline followed by cocaine, are provided in the text.

differ from that of the saline-treated controls (ANOVA, Dunnett's). In general, the washout of specific binding was a monotonically decreasing function with a long plateau between 4 and 16 hours. This is readily seen in the brain and lung data (Fig. 6, A and D). No significant differences in specific binding over the 4- to 16-hour plateau period were noted for any tissue (ANOVA, Tukey's). For brain, 10.0 $\mu\text{mol/kg}$ PD144418 occupied 60% of σ_1 receptors between 4 and 16 hours. Over this same period, lung occupancy was approximately 70%, and heart occupancy was 68–88%. Occupancy in the spleen was lower, ranging from 40 to 60%.

Figure 7 shows the corresponding temporal effects, from 15 minutes to 24 hours, of 10.0 $\mu\text{mol/kg}$ PD144418 pretreatments on locomotor hyperactivity for the 60-minute period after administration of 66 $\mu\text{mol/kg}$ cocaine. Significant attenuation was observed at 15 minutes, as well as 4 and 16 hours, with respect to the control group of animals that received only saline (ANOVA, Dunnett's). There were no significant differences in

locomotor hyperactivity between the PD144418 treatment groups from 15 minutes to 24 hours (ANOVA, Tukey's). However, a strong one-tailed Pearson correlation ($r^2 = 0.94$, $P = 0.01$) was observed for the increase in locomotor hyperactivity with respect to the time after PD144418 administration (data not shown). Figure 8 depicts the time-response relationships for PD144418 occupancy of cerebral σ_1 receptors overlaid with those for attenuation of cocaine-induced locomotor hyperactivity as percentage of control values.

Discussion

The present work addresses the apparent paradox of PD144418 being reported by Akunne et al. (1997) as a potent and selective σ_1 receptor antagonist with no ability to counteract cocaine-induced locomotor hyperactivity. In vitro binding studies, in vivo receptor occupancy studies, and behavioral studies were employed. As discussed below, the binding data

Fig. 5. (A) Dose-response relationship between PD144418 occupancy of σ_1 receptors at 30 minutes, determined from inhibition of [^{125}I]E-IA-DM-PE-PIPZE specific binding in mouse whole brain, and the attenuation of cocaine-induced locomotor hyperactivity over a 60-minute period, determined as total distance traveled for each treatment condition minus the basal activity. Data represent percentage of control values $\pm$ S.E.M., transformed from the groups described in Figs. 3A and 4A. (B) Linear regression of percentage receptor occupancy and reductions in cocaine-induced locomotor hyperactivity represented by the solid line, with the 95% confidence limits represented by the dashed lines.

confirmed that PD144418 should be considered a selective, high-affinity σ_1 receptor ligand as reported by Akunne and colleagues (1997), the *in vivo* studies provided σ_1 receptor occupancy measures for PD144418 as a function of dose and time, and the behavioral studies showed that PD144418 indeed attenuates cocaine-induced motor stimulation. Notably, cerebral σ_1 receptor occupancy by PD144418 correlated with the ligand's ability to block cocaine's behavioral effects.

The *in vitro* σ_1 receptor assays were performed at pH 7.4 and 37°C in guinea pig brain membranes, where the K_d of [^3H](+)-pentazocine is 2.3–2.9 nM (DeHaven-Hudkins et al., 1992; Cobos et al., 2005; Lever et al., 2006). Assays of Akunne et al. (1997) were performed at pH 8.0 and 25°C, where the K_d of [^3H](+)-pentazocine was reported as 4.7 nM. Under the latter conditions, steady state was likely not attained (DeHaven-Hudkins et al., 1992). As reviewed by Hulme and Trevethick (2010), use of the Cheng-Prusoff (Cheng and Prusoff, 1973) relationship for normalization of K_i values cannot fully account for subtle variations in receptor binding assays, particularly under nonequilibrium conditions. So, it is not surprising that the present σ_1 receptor K_i of 0.46 nM

differs to a degree from the K_i of 0.08 nM previously reported. Interestingly, values for haloperidol were consistent between studies. PD144418 displayed a more potent K_i of 0.19 nM against the σ_1 receptor ligand [^{125}I]E-IA-DM-PE-PIPZE, which has a K_d of 3.79 nM in mouse brain membranes (Lever et al., 2012). The σ_2 receptor K_i of 1654 nM for PD144418 determined in guinea pig brain membranes proved close to the K_i of 1377 nM reported by Akunne et al. (1997) using NG108-15 cell membranes. On the basis of K_i ratios, PD144418 shows at least 3500-fold selectivity for binding to σ_1 over σ_2 receptors. Interactions with monoamine transporters were weak, with K_i values ranging from 9 μM for DAT to $>100 \mu\text{M}$ for SERT and NET.

The regional distribution of radioactivity in mouse brain after administration of [^{125}I]E-IA-DM-PE-PIPZE was consistent with selective labeling of σ_1 receptors and showed rank order similarity to prior studies performed using the well characterized σ_1 ligand [^3H]SKF10,047. The *in vivo* data also compare well to that from an *in vitro* study by Kovács and Larson (1995), who determined the maximal binding densities (B_{max}), as femtomoles per milligram protein, of σ_1 sites labeled by [^3H](+)-pentazocine in Swiss Webster mouse cerebellum and cortex. Their cerebellum-to-cortex B_{max} ratio was 1.3, and the average cerebellum to cortex uptake ratio for [^{125}I]E-IA-DM-PE-PIPZE in the present study is 1.4. The dosage of radioligand (2.5 $\mu\text{Ci}/\text{mouse}$; 40 pmol/kg) was at least three orders of magnitude below the level identified for saturation of the sites *in vivo* (Lever et al., 2012). PD144418 effectively penetrated the central nervous system when administered intraperitoneally, and significantly blocked the uptake of [^{125}I]E-IA-DM-PE-PIPZE in all brain regions examined. In fact, PD144418 administered at 1.0 $\mu\text{mol}/\text{kg}$ i.p. was nearly as effective as a fivefold higher dosage of BD1063 administered intravenously. This is despite the enhanced first-pass metabolism that might occur with the intraperitoneal route and is in accord with the 20-fold higher affinity of PD144418 for σ_1 receptors compared with BD1063 ($K_i = 9.1$ nM; Matsumoto et al., 1995). PD144418 displayed an ED_{50} of 0.22 $\mu\text{mol}/\text{kg}$ in whole mouse brain, and ED_{50} values ranging from 0.08 to 0.39 $\mu\text{mol}/\text{kg}$ in heart, lung, and spleen. In a directly comparable study (Lever et al., 2014), BD1063 administered intraperitoneally gave an ED_{50} of 0.62 $\mu\text{mol}/\text{kg}$ in mouse brain, with ED_{50} values between 0.14 and 1.47 $\mu\text{mol}/\text{kg}$ for the heart, lung, and spleen.

A key finding of the present study is that PD144418 profiles as a behavioral antagonist that attenuates cocaine's locomotor stimulatory effects in dose-dependent fashion. Thus there is no contradiction of the established paradigm of selective σ_1 receptor antagonists blocking cocaine-induced behaviors (Menkel et al., 1991; Matsumoto et al., 2003, 2014). Considering the high σ_1 receptor affinity and selectivity of PD144418, our findings provide additional support for the role played by σ_1 receptors in psychostimulant actions. We surmise that unidentifiable methodological issues prevented Akunne and colleagues (1997) from observing attenuation of cocaine's motor stimulatory effects by either PD144418 or BMY14802. Some differences in the locomotor-activating effects of cocaine have been reported as a function of mouse age, sex, and strain (McCarthy et al., 2004). However, the present studies and those of Akunne et al. (1997) both used adult, male Swiss-type mice (CD-1 versus Swiss Webster), with similar timing for intraperitoneal cocaine and test ligand

Fig. 6. Inhibition of specific binding to σ_1 receptors in whole mouse brain (A), heart (B), spleen (C), and lung (D), 30 minutes after administration of [125 I]E-1A-DM-PE-PIPZE, by pretreatments with PD144418 (10.0 μ mol/kg i.p.) at 1 minute, as well as 4, 16, and 24 hours. Data represent mean $\pm$ S.E.M., $n = 3-7$. Nonspecific binding was defined by a separate group of animals ($n = 8$) that received BD1063 (5.0 μ mol/kg i.v.) 5 minutes before radioligand. *Significant differences from control (ANOVA, Dunnett's) ($P < 0.05$).

administrations followed by the activity measurements. We determined a behavioral ED_{50} of 0.79 μ mol/kg (0.3 mg/kg) for PD144418 attenuation of cocaine-induced locomotor stimulation, whereas Akunne et al. (1997) did not observe effects at doses up to 30-fold higher (10 mg/kg). One difference between studies is the cocaine dose employed, which was 10 mg/kg

(33 μ mol/kg) in the Akunne study and 20 mg/kg (66 μ mol/kg) in the present study. Previous dose-response profiles show a relatively small difference, on the order of 20%, in the levels of locomotor hyperactivity induced by these doses of cocaine in either Swiss Webster (Menkel et al., 1991) or CD-1 mice (Rodvelt et al., 2011). Consequently, cocaine dosage is not likely to have had a major impact.

At a practical level, the preparation of stock solutions of PD144418 oxalate in aqueous vehicles requires heat and thorough mixing. This engenders some concern regarding solubility and the potential for drug precipitation. The chemical form of PD144418, salt or free base, was not specified by Akunne et al. (1997). These investigators reported the same depression of basal locomotor activity by PD144418 that we observed, a finding that requires higher levels of PD144418 (10–30 mg/kg), and also showed antagonism of mescaline-induced scratching behaviors by PD144418 (3–30 mg/kg). Thus, Akunne and colleagues (1997) successfully prepared formulations of PD144418 for in vivo use. Nonetheless, one possible explanation for the different findings between studies is that an unnoticed solubility or precipitation issue may have compromised the data obtained by Akunne et al. (1997) from the subset of experiments on cocaine-induced hyperactivity.

With σ_1 receptor occupancy and behavioral measures in hand, we investigated relationships between fractional occupancy and PD144418 actions. Pharmacokinetic aspects of in vivo radioligand binding and locomotor activity studies were

Fig. 7. Attenuation of locomotor hyperactivity induced by cocaine (66 μ mol/kg i.p.) over the 60-minute period after drug administration, by pretreatments with PD144418 (10.0 μ mol/kg i.p.) at 15 minutes, as well as 4, 16, and 24 hours. *Significant differences from the saline-cocaine-treated control group (ANOVA, Dunnett's) ($P < 0.05$). Data represent mean $\pm$ S.E.M., $n = 9-11$.

Fig. 8. Time-response relationships for PD144418 (10.0 $\mu\text{mol/kg}$ i.p.) pretreatments on occupancy of σ_1 receptors, determined from inhibition of [^{125}I]E-1A-DM-PE-PIPZE specific binding in mouse whole brain, and attenuation of cocaine-induced locomotor hyperactivity over a 60-minute period, determined as total distance traveled for each treatment condition minus basal activity. Values are percentage of control $\pm$ S.E.M., transformed from the data sets described in Figs. 6A and 7.

constructed so receptor occupancy was assessed 30 minutes after administration of PD144418, which also corresponds to the time of cocaine's maximal effects on locomotor activity. As occupancy increased, cocaine's ability to stimulate locomotor activity decreased. PD144418 displayed an ED_{50} of 0.22 $\mu\text{mol/kg}$ for σ_1 receptor occupancy in whole mouse brain and a behavioral ED_{50} of 0.79 $\mu\text{mol/kg}$. About 80% σ_1 receptor occupancy was associated with the 50% reduction in cocaine's ability to stimulate motor activity. At 95% occupancy (10 $\mu\text{mol/kg}$), about 70% reduction in locomotor stimulation was observed. These data indicate that limited σ_1 receptor reserve exists for modulation of this cocaine-induced behavior. Further depression of cocaine-induced motor activity, to 85% of saline control levels, when PD144418 was used at 31.6 $\mu\text{mol/kg}$ cannot easily be attributed to increased σ_1 receptor occupancy. The phenomenon likely reflects an additional contribution from a general impairment of motor activity, by an unknown mechanism, when the ligand is used at this dosage (Figs. 4A and 5A). Suppression of basal locomotor activity in mice by PD144418 at doses ≥ 10 mg/kg is a consistent finding between the present study and the original work of Akunne et al. (1997). Attenuation of basal activity is not a characteristic feature of σ_1 receptor antagonism, but sedative effects have been observed for certain selective σ_1 receptor ligands (Kaushal et al., 2011).

From the viewpoint of medications development, an open question is the level and duration of σ_1 receptor occupancy needed to foster anticocaine effects. To address this topic, PD144418 was administered from 15 minutes to 24 hours before cocaine, and receptor occupancy and effects on cocaine-induced locomotor hyperactivity were both determined. Similar approaches have been used to investigate relationships between behavioral effects and the *in vivo* occupancy of a variety of recognition sites, including cannabinoid receptors (Gifford et al., 1999), dopamine D_2 receptors (Wadenberg et al., 2000), the DAT (Desai et al., 2005), histamine H_3 receptors (Le et al., 2008), and corticotropin releasing factor type-1 receptors (Ramsey et al., 2011), but have not been previously applied to the study of behavioral effects thought to be mediated by σ_1 receptors.

The 15-minute pretreatment studies indicate that 80% occupancy of σ_1 receptors by a potent antagonist ligand, such as PD144418, is needed to attenuate cocaine-induced locomotor hyperactivity by 50%. PD144418 effects were not measured at occupancy levels between 20 and 80%, so a gradual induction would not be readily apparent from the present work. However, the overall findings suggest that 80% σ_1 receptor occupancy may well be a critical threshold. For duration studies, a PD144418 dosage of 10 $\mu\text{mol/kg}$ was selected because σ_1 receptor occupancy would start at 95%, maximal hyperactivity reduction would be about 70%, there would be no confounding effects on basal activity, and the DAT would not be occupied by PD144418 or possible metabolites. With a 4-hour pretreatment, receptor occupancy decreased to 62%, whereas attenuation of hyperactivity remained near the maximal 70%. With 16-hour pretreatment, receptor occupancy was steady at 60%, whereas hyperactivity was reduced by 45%. Because σ_1 receptor occupancy is the same at 4 and 16 hours, whereas attenuation of hyperactivity is trending lower, one might speculate that downstream effects diminish with time. After the 24-hour pretreatment, low receptor occupancy was observed and effects on hyperactivity were negligible. We conclude that σ_1 receptor occupancy by antagonists correlates directly with their ability to attenuate cocaine's behavioral effects. Furthermore, sustained occupancy of σ_1 receptors by potent and selective antagonists can mitigate cocaine's stimulatory effects well in advance of exposure to the drug.

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Authorship Contributions

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