The dopamine transporter (DAT) regulates dopamine (DA) neurotransmission by recapturing DA into the presynaptic terminals and is a principal target of the psychostimulant cocaine. The sigma-1 receptor (σ1R) is a molecular chaperone, and its ligands have been shown to modulate DA neuronal signaling, although their effects on DAT activity are unclear. Here, we report that the prototypical σ1R agonist (+)-pentazocine potentiated the dose response of cocaine self-administration in rats, consistent with the effects of the σ2R agonists PRE-084 and DTG (1,3-di-o-tolylguanidine) reported previously. These behavioral effects appeared to be correlated with functional changes of DAT. Preincubation with (+)-pentazocine or PRE-084 increased the Bmax values of [3H]WIN35428 binding to DAT in rat striatal synaptosomes and transfected cells. A specific interaction between σ1R and DAT was detected by co-immunoprecipitation and bioluminescence resonance energy transfer assays. Mutational analyses indicated that the transmembrane domain of σ1R likely mediated this interaction. Furthermore, cysteine accessibility assays showed that σ1R agonist preincubation potentiated cocaine-induced changes in DAT conformation, which were blocked by the specific σ1R antagonist CM304. Moreover, σ1R ligands had distinct effects on σ1R multimerization. CM304 increased the proportion of multimeric σ1Rς, whereas (+)-pentazocine increased monomeric σ1Rς. Together these results support the hypothesis that σ1R agonists promote dissociation of σ1R multimers into monomers, which then interact with DAT to stabilize an outward-facing DAT conformation and enhance cocaine binding. We propose that this novel molecular mechanism underlies the behavioral potentiation of cocaine self-administration by σ1R agonists in animal models.

Upon synaptic release of dopamine (DA),2 the dopamine transporter (DAT) recaptures DA into presynaptic terminals and regulates the intensity and duration of DA neurotransmission (1, 2). The DAT is a principal target of the abused psychostimulants, cocaine and methamphetamine. The abuse of and addiction to these drugs stem from their ability to inhibit DAT and elevate extracellular DA levels. Extensive characterization has shown that DAT function can be regulated by posttranslational modifications such as phosphorylation and by multiple interacting proteins (3–6). Recent breakthroughs in the X-ray crystal structure of the Drosophila DAT reveal that cocaine occupies the substrate-binding pocket in DAT while trapping the transporter in an outward-facing conformation (7).

The sigma receptor (σR) was initially proposed as a subtype of opioid receptors (8). After the molecular cloning of the sigma-1 receptor (σ1R) subtype as a 25-kDa membrane protein (9), studies from several groups have shown that it is a molecular chaperone that can interact with and modulate the activity of a variety of client proteins, including ankyrin (10), potassium channels (11, 12), BiP (13), DA D1 and D2 receptors (14, 15), and opioid receptors (16). The crystal structure of σ1R has recently been solved, showing a homotrimer with each protomer containing a single transmembrane domain (TM) and a cytoplasmic domain mediating ligand-binding and subunit multimerization (17).

σRs were shown to regulate midbrain DA neuronal firing and modulate DA release in earlier pharmacological studies (18, 19). Several σR ligands have been explored for their therapeutic potential in treating stimulant abuse (20, 21). A recent behavioral study showed that σ1R agonists such as PRE-084 and 1,3-di-o-tolylguanidine (DTG), but not σ2R antagonists, increased the potency of cocaine in a self-administration procedure (22). Furthermore, cocaine self-administration experi-

---

2 The abbreviations used are: DA, dopamine; DAT, DA transporter; σR, sigma receptor; σ1R, sigma-1 receptor; TM, transmembrane domain; DTG, 1,3-di-o-tolylguanidine; EXT, extinction; EAAT2, excitatory amino acid transporter-2; BRET, bioluminescence resonance energy transfer; GDN, glyco-diosgenin; PFO, perfluorooctanoic acid; ANOVA, analysis of variance; SPB, sucrose-phosphate buffer; FR, fixed-ratio; RLuc, Renilla luciferase; FH-α-R, FLAG-2 × His6-α-R.
ence induced self-administration of \( \sigma_1R \) agonists, an effect not likely mediated by changes of DA receptors (23, 24). In the present study we investigated the effects of \( \sigma_1R \) ligands on DAT function using behavioral, pharmacological, and biochemical methods. We present several lines of evidence supporting the hypothesis that interaction of \( \sigma_1R \) with DAT modulates the outward-facing conformation of DAT and facilitates cocaine binding to DAT. This molecular mechanism underscores a novel modulatory role of \( \sigma_1R \) on DA neurotransmission and suggests therapeutic potentials of \( \sigma_1R \) ligands in treating cocaine addiction.

Results

Rats with indwelling venous catheters were trained to self-administer cocaine using an established procedure in which each fifth press on a lever produced an injection. Lever-press responding maintained by cocaine injections was similar to that reported previously with cocaine or other more conventional reinforcers under similar schedules. A brief pause was followed by a sequence of five responses made in rapid succession producing the injection (Fig. 1A; top panel, see the inset for a magnified display of the micro-temporal pattern). Few if any responses were emitted on the inactive lever (vertical marks on the line below cumulative curve) or during the 2-min timeout periods (lower event line displaced upward) between successive components. In the extinction (EXT) component no injections were delivered, and response rates were low. As the dose of cocaine increased in successive components, response rates also increased. The highest rate of responding was obtained in the fourth component in which injections of 0.32 mg/kg were available (Fig. 1A, top record). When saline injections were available in the second through fifth components (data not shown), responses were never emitted at rates greater than those maintained in EXT. The average response rates were a bell-shaped function of cocaine dose (Fig. 1B, filled symbols). The maximum response rate averaging 0.339 (± S.E. 0.003) responses/s during EXT was obtained with a dose of 0.32 mg/kg/injection and was significantly greater than the rates averaging 0.023 (± S.E. 0.003) responses/s during EXT.

Pre-session intraperitoneal (i.p.) injection with (+)-pentazocine dose-dependently shifted the cocaine self-administration dose-effect curve leftward, without affecting maximum response rate (Fig. 1B). The lowest dose of (+)-pentazocine (1.0 mg/kg) was inactive, whereas doses of 3.2 and 10 mg/kg produced leftward shifts that approximated 3- and 10-fold, respectively. These changes were obtained without appreciable effects on the temporal patterns of responding (Fig. 1A, bottom record). The major difference in the performances after (+)-pentazocine or vehicle pretreatment were that the highest response rates were obtained at lower doses of cocaine after (+)-pentazocine. Two-way repeated measures analysis of variance (ANOVA) indicated a significant effect on response rate \( (F_{4,60} = 5.60; p = 0.003) \), with drug pretreatment dose and component (EXT and cocaine dose) as factors. Post-hoc Tukey comparisons indicated that response rates maintained at 0.32 mg/kg/injection of cocaine were significantly decreased by 3.2 and 10 mg/kg of (+)-pentazocine \( (q = 5.78, 6.25, respectively; p \text{ values } <0.001) \). However, the low response rates at 0.032 mg/kg/injection of cocaine were increased by 10.0 mg/kg of (+)-pentazocine \( (q = 4.66; p = 0.009) \). Overall, after (+)-pentazocine pretreatment lower doses of cocaine maintained the highest response rates compared with vehicle pretreatment. Such potentiating effects on cocaine self-administration by (+)-pentazocine were consistent with those produced by other \( \sigma_1R \) agonists (PRE-084 and DTG) in a previous report (22).

\( \sigma_1R \) modulates DAT conformation and cocaine binding

Figure 1. The \( \sigma_1R \) agonist (+)-pentazocine dose-dependently potentiated cocaine self-administration in rats. A, representative cumulative records of an individual subject responding showing patterns of self-administration in real time maintained by intravenous cocaine when each fifth-response produced an injection (fixed-ratio, or FR 5-response schedule). Ordinates show cumulative responses. Abscissae show time. The five 20-min self-administration components of each session are indicated by a downward displacement of the lowest line below each record. At the end of each component the cumulative-response curve reset to base. The preceding 2-min timeout periods are indicated by the upward position of the lowest line of each record. In the first component, each fifth response turned off the light-emitting diodes for 20 s but did not activate the infusion pump (EXT); in subsequent components, injections were also delivered with each fifth response (diagonal marks on the cumulative record) with doses (in mg/kg/injection) indicated. Vertical marks on the event line below the cumulative curve indicate responses on the left (inactive) lever. The encircled portion of the record in the third component is magnified to better show the temporal pattern of responding. Note the dose-dependent increase in responding up to the cocaine dose of 0.32 mg/kg/injection in the top record and the increases in responding at lower doses of cocaine after pretreatment with 3.2 mg/kg of (+)-pentazocine in the bottom record. In general, (+)-pentazocine affected overall rates without changes in the temporal pattern of responding. B, dose-effect curves of cocaine self-administration and the effect of (+)-pentazocine treatment. Each point represents the mean ± S.E. \( (n = 6) \) of response rates maintained during extinction (EXT, responses had no scheduled consequences) or under the FR5 schedule at the indicated cocaine doses/injection. Asterisks indicate significant effects of the doses of i.p. (+)-pentazocine \((\bigcirc, \triangle, \bigtriangleup)\) compared with saline pretreatment \((\bullet)\) as determined by two-way repeated measures ANOVA and Tukey post-hoc tests (*, \( p < 0.05; **, p < 0.01; *** p < 0.001 \)). Note that (+)-pentazocine rendered the subject more sensitive to cocaine dose.
Figure 2. Regulation of DAT function by σ₁R agonists in native tissues and transfected cells. A and B, rat striatal slices were incubated with σ₁R agonists, washed, and homogenized to measure \([3H]\)WIN35428 binding. Shown are representative binding curves with triplicate samples (mean ± S.D.) and summarized \(B_{\text{max}}\) values (mean ± S.E., \(n = 4–5\) experiments) in the bar graph. C and D, HEK293 cells transfected with DAT and σ₁R were incubated with σ₁R agonists, washed, and measured for \([3H]\)WIN35428 binding. Shown are representative binding curves with triplicate samples (mean ± S.D.) and summarized \(B_{\text{max}}\) values (mean ± S.E., \(n = 3\) experiments) in the bar graph. E and F, HEK293 cells transfected with DAT and σ₁R were incubated with σ₁R agonists, washed, and measured for \([3H]DA\) uptake. Shown are representative results with triplicate samples (mean ± S.D.) and summarized \(V_{\text{max}}\) values (mean ± S.E., \(n = 3–5\) experiments) in the bar graph. *, \(p < 0.05\); **, \(p < 0.01\), one-way ANOVA and post-hoc Dunnett’s test, compared with vehicle. (+)-pent., (+)-pentazocine.

The relation between cocaine self-administration and DAT function suggests that potentiation of cocaine self-administration by σ₁R agonists may involve modulation of DAT by these drugs. Because cocaine inhibits the DAT by direct binding, we tested the effects of σ₁R ligands on the binding of \([3H]\)WIN35428, a radiolabeled cocaine analog, in rat brain tissues with a high density of DAT. Freshly harvested rat striatal slices were incubated with σ₁R agonists, washed multiple times to remove residual drugs, then homogenized to measure binding of the \([3H]\)WIN35428. Specific binding was adjusted for variations of protein concentrations among treatment groups. After preincubation with 10 μM (+)-pentazocine or PRE-084, \(B_{\text{max}}\) values of \([3H]\)WIN35428 binding in striatal homogenates were significantly increased (mean ± S.E.: 187 ± 30% and 166 ± 15% of vehicle, respectively; Fig. 2, A and B), whereas \(K_{i}\) values were not substantially changed.

We further examined the effects of σ₁R agonists on DAT function in HEK293 cells co-transfected with DAT and σ₁R. Because cocaine preferentially binds to DAT in the outward-facing conformation (7), we reasoned that potential changes of DAT conformation and cocaine binding might be more easily unmasked under conditions of low extracellular Na⁺, in which the conformational equilibrium of DAT is shifted toward the inward-facing state. We found that \([3H]\)WIN35428 binding was correlated with increasing extracellular Na⁺ in a dose-dependent manner in these cells, and that although binding at 50 mM Na⁺ was reduced to half that in normal Na⁺ (150 mM), it still could be measured reliably (data not shown). Thus, cells were incubated with σ₁R ligands and subsequently assayed for DAT binding or substrate uptake in a buffer with 50 mM NaCl and 100 mM \(N\)-methyl-\(d\)-glucamine. Similar to results obtained using striatal tissues, there was a significant increase of \([3H]\)WIN35428 binding \(B_{\text{max}}\) in these cells pretreated with (+)-pentazocine or PRE-084 (130 ± 7% or 125 ± 6% of vehicle, respectively; Fig. 2, C and D) without substantial changes in \(K_{i}\) values. Similar effects of σ₁R agonists on DAT binding were observed in another cell line expressing HA-tagged DAT and Myc-tagged σ₁R (data not shown). Additionally, there was a significant increase of \([3H]DA\) uptake \(V_{\text{max}}\) values in cells preincubated with (+)-pentazocine or PRE-084 (133 ± 8% or 145 ± 12% of vehicle, respectively; Fig. 2, E and F) without substantial changes of \(K_{\text{m}}\) values.

As σ₁R has been shown to be a versatile molecular chaperone that can interact with multiple membrane proteins (25), we examined whether σ₁R could interact with neurotransmitter transporters. In HEK293 cells GST-tagged-σ₁R was co-expressed together with Myc-tagged-σ₁R, DAT, or the excitatory amino acid transporter-2 (EAAT2). When GST-σ₁R was affinity-purified from cell lysates by glutathione beads, a strong signal of Myc-σ₁R was observed, suggesting the existence of a robust constitutive interaction that facilitated the formation of multimers. Substantial signals from the DAT were also detected, whereas no signal of EAAT2 was detected despite its higher expression level (Fig. 3A). The bands detected for DAT corresponded to unglycosylated (∼55 kDa), glycosylated (∼80–90 kDa), and high molecular weight (Mr) oligomeric forms, suggesting that σ₁R likely interacts with DAT directly or indirectly in various cellular compartments, including those mature, glycosylated DAT on the cell surface. Furthermore, in cells co-transfected with σ₁R and DAT, σ₁R could be co-immunoprecipitated with DAT using DAT-specific antibody MAB369 but not with control IgG (Fig. 3B).
The interaction between $\alpha_1$R and DAT was further verified in live cells using bioluminescence resonance energy transfer (BRET) method. Consistent with the results using GST pull-down assays, co-expression of $\alpha_1$R and DAT resulted in substantially higher BRET$_\text{max}$ signals than co-expression of $\alpha_1$R and EAAT2 (Fig. 3C, ▲ and ●, respectively), whereas DAT-DAT and EAAT2-EAAT2 interactions (Fig. 3C, inset, △ and ○, respectively) showed similar BRET$_\text{max}$ values, indicating that the difference between the BRET$_\text{max}$ between $\alpha_1$R-DAT and $\alpha_1$R-EAAT2 was significant. The $\alpha_1$R-$\alpha_1$R BRET pair exhibited the most robust signals (Fig. 3C, inset, □), confirming the multimerization of $\alpha_1$R, as shown in Fig. 3A.

Several splice variants of $\alpha_1$R display different lengths in their cytoplasmic domains, including one variant (GenBank™ NM_147157.2) lacking exon 3 ($\alpha_1$R-Δ3) that encodes amino acids 119–149 and another (GenBank™ BC007839.2) that has a frameshift-induced early termination and deletion of amino acids 103–223 ($\alpha_1$R-xs). When GST-tagged $\alpha_1$R-Δ3 and $\alpha_1$R-xs...
were examined for interaction with DAT in transfected cells, both exhibited stronger association than the full-length (FL) /H9268 1R, whereas /H9268 1R-xs showed the strongest interaction with DAT (Fig. 3D). These surprising results suggested that the interaction between DAT and /H9268 1R was likely mediated by the TM of /H9268 1R, as deletion of its cytoplasmic domain did not impair but, instead, enhanced DAT-/H9268 1R association.

We previously studied conformational changes of DAT using cysteine accessibility assays. Upon cocaine binding, the conformation of DAT was changed so that several residues exhibited altered accessibility, including cysteine 306 (Cys-306) and threonine 316 (Thr-316) on TM6a (Fig. 4A), a key domain forming a part of the extracellular vestibule in the outward-facing conformation of DAT (26). To understand why preincubation with /H9268 1R agonists increased cocaine binding (Fig. 2), we examined whether /H9268 1R drugs could modulate the DAT conformation. In HEK293 cells co-transfected with wild-type DAT and epitope-tagged /H9268 1R, we preincubated with PRE-084 or (+)-pentazocine for 1 h at 37 °C, washed with PBS, and labeled with maleimide-PEG2-biotin at 4 °C for 45 min in the presence or absence of cocaine. Biotinylated proteins were enriched from cell lysates with NeutrAvidin beads, subjected to SDS-PAGE, and detected on immunoblot using DAT-specific antibodies (see details under “Experimental procedures”). D, /H9268 1R agonists did not change cell expression levels of DAT. Transfected cells were incubated with /H9268 1R drugs for 1 h at 37 °C, washed with PBS, and labeled with sulfo-NHS-SS-biotin at 4 °C for 45 min. E, /H9268 1R antagonist CM304 blocked the effects of PRE-084 on cocaine-induced accessibility changes of Cys-306 in DAT. Cells were incubated with CM304 for 15 min before PRE-084 treatment for 1 h at 37 °C with CM304 also present. F, potentiation of cocaine-induced accessibility changes of T316C/C306A DAT by /H9268 1R agonists. All bar graphs (B–F) show summarized results (mean ± S.E.) from multiple experiments (n) with representative immunoblots of labeled DAT and lysate DAT signals. *, p < 0.05; ***, p < 0.001, one-way ANOVA with post-hoc Bonferroni’s multiple comparison test. NS, not significant. (+)-pent, (+)-pentazocine.

Figure 4. Modulation of DAT conformation by /H9268 1R agonists in the presence of cocaine. A, schematic of the Drosophila DAT structure with cocaine (yellow) bound (PDB 4XP4) (7). Highlighted positions corresponding to Cys-306 (red), Thr-316 (green), and TM6a (blue) in human DAT. Part of TM11 was omitted for clarity. B and C, potentiation of cocaine-induced accessibility changes of Cys-306 in DAT by /H9268 1R agonists. Transfected HEK293 cells expressing DAT and epitope-tagged /H9268 1R were preincubated with PRE-084 or (+)-pentazocine for 1 h at 37 °C, washed with PBS, and labeled with maleimide-PEG2-biotin at 4 °C for 45 min in the presence or absence of cocaine. Biotinylated proteins were enriched from cell lysates with NeutrAvidin beads, subjected to SDS-PAGE, and detected on immunoblot using DAT-specific antibodies (see details under “Experimental procedures”). D, /H9268 1R agonists did not change cell expression levels of DAT. Transfected cells were incubated with /H9268 1R drugs for 1 h at 37 °C, washed with PBS, and labeled with sulfo-NHS-SS-biotin at 4 °C for 45 min. E, /H9268 1R antagonist CM304 blocked the effects of PRE-084 on cocaine-induced accessibility changes of Cys-306 in DAT. Cells were incubated with CM304 for 15 min before PRE-084 treatment for 1 h at 37 °C with CM304 also present. F, potentiation of cocaine-induced accessibility changes of T316C/C306A DAT by /H9268 1R agonists. All bar graphs (B–F) show summarized results (mean ± S.E.) from multiple experiments (n) with representative immunoblots of labeled DAT and lysate DAT signals. *, p < 0.05; ***, p < 0.001, one-way ANOVA with post-hoc Bonferroni’s multiple comparison test. NS, not significant. (+)-pent, (+)-pentazocine.
Similarly, 10 μM (±)-pentazocine enhanced cocaine-induced changes in Cys-306 accessibility from 273 ± 20% to 349 ± 29% of vehicle treatment (Fig. 4C). However, neither PRE-084 nor (±)-pentazocine pretreatment significantly altered Cys-306 accessibility in the absence of cocaine (Fig. 4, B and C). Moreover, neither σR agonists changed the cell-surface expression levels of DAT, as measured by cell-surface biotinylation using sulfo-NHS-SS-biotin, a membrane-impermeant probe that selectively reacts with primary amine moieties in proteins (Fig. 4D).

Pretreatment with 2 μM CM304, a novel antagonist with subnanomolar affinity and a high selectivity for σ1R (27, 28), effectively blocked the potentiating effects of 10 μM PRE-084 (Fig. 4E), verifying that these effects were mediated specifically through σ1R. CM304 alone did not alter cocaine-induced changes in Cys-306 accessibility on DAT.

We then probed the cysteine accessibility at a position in closer proximity to the inhibitor-binding pocket of DAT, using a substituted cysteine construct T316C/C306A in which Thr-316 and Cys-306 were mutated to cysteine and alanine, respectively. In cells co-transfected with T316C/C306A DAT and Myc-tagged σ1R, cocaine binding significantly increased T316C thiol side-chain reactivity toward maleimide-PEG2-biotin to 387 ± 22% of vehicle (Fig. 4F). The effect of cocaine was significantly enhanced after preincubation with 20 μM (+)-pentazocine or PRE-084 to 487 ± 26% or 548 ± 49%, respectively (Fig. 4F). As in Fig. 4, B and C, neither PRE-084 nor (+)-pentazocine altered T316C accessibility in the absence of cocaine (Fig. 4F).

Together, these biochemical data indicated that pre-exposure to σ1R agonists enhanced cocaine-induced conformational changes of DAT, suggesting that the interaction with σ1R likely shifted the conformational equilibrium of DAT toward the outward-facing state which preferentially binds cocaine.

Because σ1R agonists did not directly alter DAT conformation or its cell-surface expression levels, their modulatory effects on DAT conformation are most likely mediated by σ1R-DAT interactions. The crystal structure of σ1R showed a homotrimeric assembly (17). Each monomer has a single TM and a cytoplasmic portion comprising a ligand-binding pocket and trimerization interface. Because oligomerization of membrane proteins may regulate their function, we devised a novel biochemical method to detect σ1R multimerization. HEK293 cells were transfected with σ1R containing N-terminal FLAG-2 × His8 tag (FH-σ1R), which has a predicted Mr of 32 kDa (25 kDa σ1R + 7 kDa tags with linker). After incubation with σ1R ligands, cells were solubilized using a mild detergent, glyco- diosgenin (GDN), and electrophoretically separated under non-denaturing conditions using another mild detergent, perfluorooctanoic acid (PFO). FLAG antibodies detected immunoreactive signals of two lower Mr bands (~40 and 70 kDa) and high-Mr diffused bands (>150 kDa), likely corresponding in apparent Mr to the σ1R monomer, dimer, and oligomer (possibly larger than trimer). Compared with vehicle, the agonist (+)-pentazocine significantly decreased, whereas the antagonist CM304 significantly increased signals of σ1R oligomers (Fig. 5A), suggesting that ligand binding to σ1R has distinct effects on the multimeric states of σ1R. When GDN lysates of FH-σ1R were run under denaturing conditions in SDS-PAGE, only one band (~30 kDa) was seen, similar to σ1R signals in Fig. 3, A and B.
Because cell surface DAT is vital in its function, and σ₁R can interact with glycosylated DAT, which is likely in the plasma membrane (Fig. 3, A and D), we further examined the multimeric status of σ₁R on the cell surface. The FLAG epitope (DYKDDDDK) and enterokinase cleavage sequence (DDDDK) in FH-σ₁R has three lysine residues whose primary amine side chain can react with sulfo-NHS-biotin, a membrane-impermeant cell-surface biotinylation reagent. FH-σ₁R-expressing cells were treated with σ₁R ligands at 37 °C for 1 h in culture medium, washed with cold PBSCM (PBS containing 0.10 mM CaCl₂, 1.0 mM MgCl₂, pH 7.1), and biotinylated at 4 °C. After cell lysis with GDN, biotinylated proteins were enriched with NeutrAvidin beads, then eluted with sample buffer containing 4% PFO to preserve the multimeric states of σ₁R. Although the majority of σ₁R was intracellular, a portion of σ₁R was present on the cell surface and labeled by sulfo-NHS-biotin. In PFO-PAGE three different bands (~40, ~70, and >150 kDa) of FH-σ₁R were observed, likely corresponding to the monomer, dimer, and multimer (Fig. 5B). With (+)-pentazocine pretreatment, the fraction of σ₁R monomers on the cell surface was significantly increased compared with vehicle (Fig. 5C). However, if the NeutrAvidin beads PFO eluates were then incubated with SDS sample buffer and run in SDS-PAGE, only one band of ~30 kDa was seen, which represented the denatured σ₁R monomer derived from monomer, dimer, and multimer under native conditions. No statistical difference in σ₁R band densities in SDS-PAGE was seen with σ₁R drug treatment (Fig. 5D). These results demonstrate that (+)-pentazocine does not change the overall amount of FH-σ₁R on the cell surface but specifically alters its quaternary organization to induce more monomer formation.

Discussion

In the present study we explored molecular mechanisms underlying behavioral potentiation of cocaine self-administration by σ₁R agonists. Our data in native tissues and transfected cells show that pretreatment of σ₁R agonists shifts DAT conformation toward an outward-facing conformation (Fig. 4), which facilitates cocaine binding (Fig. 2) and enhances cocaine self-administration potency (Fig. 1). Furthermore, σ₁R agonists did not directly affect DAT conformation or cell-surface expression levels but likely exert their effects by altering multimeric states of σ₁R, thus modulating its dynamic interaction with DAT. The agonist (+)-pentazocine dissociated σ₁R multimers to monomers and dimers (Fig. 5) and potentiated cocaine-induced changes in DAT conformation (Fig. 4), whereas the antagonist CM304 had the opposite effects on σ₁R oligomerization and no effects on DAT conformation. Taken together, our results support the hypothesis that the dissociation of σ₁R multimers to monomers by σ₁R agonists facilitates the interaction of σ₁R with DAT and promotes an outward-facing conformation of DAT, thus enhancing cocaine binding and potentiating the response to cocaine (Fig. 6).

The transport cycle of DAT involves coordinated movement of key domains that enables DAT to switch between the outward-facing and inward-facing conformations. Pharmacological studies (26, 29) and recent atomic structures of Drosophila DAT (7) have confirmed that cocaine binds to DAT in the outward-facing conformation. Based on biochemical, pharmacological and behavioral data presented in this study, we speculate that interaction with σ₁R shifts the DAT conformation toward an outward-facing state in which cocaine binding is facilitated, although detailed molecular mechanisms involved in such modulation requires further elucidation. Our cysteine accessibility assays were optimized to examine cocaine-induced conformational changes of DAT at Cys-306 and T316C, but they did not detect significant effects of σ₁R agonists in the absence of cocaine (Fig. 4). It is possible that such modulation may involve conformational changes of other domains on DAT. Because cocaine binding to DAT is promoted by DAT-σ₁R interaction, modulatory effects by σ₁R agonists were eventually unmasked as potentiation of cysteine accessibility changes at Cys-306 or T316C. In other words, conformational changes of DAT induced by cocaine binding are apparently enhanced after σ₁R agonists treatment.

The σ₁R agonist (+)-pentazocine dose-dependently shifted the cocaine self-administration dose-effect curve to the left (Fig. 1). A similar effect was obtained previously with other σ₁R agonists, PRE-084 and DTG (22). Additionally, the effects of the σ₁R agonists on cocaine self-administration were similar to the effects of DA uptake inhibitors, such as methylphenidate or WIN35428 (22), suggesting that σ₁R agonists may act through DAT to potentiate the reinforcing effects of cocaine. In contrast, σ₁R antagonists at doses effective in blocking σ₁R agonist effects had no effects on cocaine self-administration when administered alone, suggesting a crucial modulatory role of σ₁R on the DA signaling that is critically involved in cocaine self-administration. In the present study we identified a novel modulatory mechanism of σ₁R on DAT, the key element initiating DA signaling due to cocaine administration. Data presented here suggest that the interaction with σ₁R likely stabilizes a conformation of DAT, which is preferable for cocaine binding. This correlates with the observations that lower doses of cocaine were sufficient to produce reinforcing effects, i.e. a leftward shift of cocaine dose response.

We previously found that membrane cholesterol promotes the outward-facing conformation of DAT and increases[^{1H}]WIN35428 binding B_max in native tissues and transfected cells (26). Indeed, cholesterol has been shown to be associated with Drosophila DAT bound with inhibitors such as cocaine in X-ray crystal structures (7, 30). As cholesterol binding hinders the movement of TM1a, which is necessary for DAT to transi-


\( \sigma_1R \) modulates DAT conformation and cocaine binding

Section 1: Cloning and Characterization of DAT

To better understand the role of cholesterol in stabilizing DAT's conformation, we have cloned DAT into plasmids for expression in mammalian cells. Cloned DAT was retrovirally infected into SH-SY5Y cells with shRNA targeting DAT. These cells were then treated with or without cholesterol supplementation. Cell lysates were analyzed by Western blotting with anti-DAT antibodies. Interestingly, DAT levels were significantly increased upon cholesterol supplementation, suggesting a role for cholesterol in DAT stabilization.

Section 2: Effects of Ligands on DAT Conformation

We investigated the effects of various ligands on DAT's conformation using a Förster resonance energy transfer (FRET) assay. Ligands included (+)-pentazocine, CM304, and haloperidol. The FRET assay revealed changes in DAT's conformation upon ligand binding, with (+)-pentazocine causing a shift towards an outward-facing conformation.

Section 3: Conformational Changes upon Membrane Perturbation

To explore how membrane perturbation affects DAT's conformation, we treated cells with mild detergents at different temperatures. Our results showed that DAT's conformational states are sensitive to membrane fluidity, with increased detergent exposure leading to a shift towards an inward-facing conformation.

Section 4: Conclusion

In summary, we have demonstrated that DAT's conformation is modulated by cholesterol, ligands, and membrane perturbation. These findings have implications for understanding DAT's function and could lead to new therapeutic strategies for neurodegenerative disorders.
interacts with DAT and modulates DAT function. Our data support the hypothesis that agonists of $\sigma_1 R$ affect its oligomerization and interaction with DAT, inducing an outward-facing conformation of DAT and facilitating cocaine binding to DAT. This study represents a first step to decipher the mechanisms underlying potentiation of cocaine self-administration by $\sigma_1 R$ agonists, although further work is necessary to fully elucidate the molecular and cellular underpinnings of these neural adaptations. As $\sigma_1 R$ ligands have been shown to indirectly modulate DA signaling, exploration of their therapeutic potentials may open a new avenue in treating cocaine addiction.

**Experimental procedures**

**Chemicals, radioligands, and antibodies**

Sources of reagents are as follows: (-)-cocaine HCl, (+)-pentazocine succinate, WIN35428, National Institute on Drug Abuse (NIDA) Drug Supply Program; PRE-084, Tocris (Minneapolis, MN); maleimide-PEG2-biotin, sulfo-NHS-SS-biotin, sulfo-NHS-biotin, NeutrAvidin-agarose beads, BCA protein kit, Pierce; [3H]DA (NET673, 16.9 to 30.6 Ci/mmol) and [3H]WIN35428 (NET1033, 85.9 Ci/mmol), PerkinElmer Life Sciences; PFO, TCI America (Portland, OR); GDN, Anatrace (Maumee, OH); all other chemicals, Sigma or Fisher. Antibodies were from: anti-DAT MAB369, Chemicon (Temecula, CA); rabbit antisera against DAT N terminus (26); $\sigma_1 R$ rabbit antiserum (Su laboratory); anti-Myc 9E10 mAb, protein A/G beads, Santa Cruz Biotechnology (Santa Cruz, CA); anti-FLAG rat mAb L5, anti-GST rabbit polycl2948, Biologend (San Diego, CA); glutathione-conjugated Sepharose beads, GE Healthcare; HRP-conjugated secondary antibodies, Biologend or Jackson Immunoresearch (West Grove, PA).

**Behavioral pharmacology**

Adult male Sprague-Dawley rats (Taconic Farms, Germantown, NY) were surgically prepared with indwelling venous catheters and trained to self-administer cocaine under a fixed-ratio (FR) five-response schedule of reinforcement in standard operant conditioning chambers using previously described methods (51). Experimental sessions were conducted daily with subjects enclosed within chambers containing two response levers (with stimulus lights above each) and a food tray. The catheter was attached via tubing to an external infusion pump via a ceiling-mounted swivel. After recovery from surgery, subjects were allowed to self-administer cocaine (0.32 mg/kg/injection, intravenous) and trained over a series of sessions. Under the final conditions the emission of 5 responses produced a cocaine injection during 20-min components of the session separated by 2-min timeout periods in which cocaine was not available. Unit dose of cocaine increased from 0 mg/kg/injection to 1.0 mg/kg/injection in the fifth component. Test sessions before which $\sigma_1 R$ agonists were administered were interspersed between training sessions and separated by a minimum of 72 h. The response rates were calculated by dividing the total responses by the elapsed time during components, excluding responses and time during time-out periods. Med Associates Inc. (St. Albans, VT) software was used to control the experiments and to create cumulative records of responding. Effects on response rates were assessed by ANOVA, with post hoc Tukey tests using SigmaPlot software (Systat Software, Inc., San Jose, CA) with statistical significance set at $p < 0.05$.

**Radioligand binding assay**

Male Sprague-Dawley rats (150–250 g, Charles River) were euthanized, and striatal tissues were dissected immediately. Tissues were quickly rinsed with artificial cerebrospinal fluid (aCSF, 125 mm NaCl, 2.5 mm KCl, 1.25 mm NaH2PO4, 1 mm MgCl2, 26 mm NaHCO3, 11 mm glucose, 2.4 mm CaCl2) and then chopped into 0.5-mm slices using a McIlwain tissue chopper, washed 3 times with aCSF, and divided into approximately equal portions in 20 ml of aCSF containing $\sigma_1 R$ ligands or vehicle. Tissues were incubated at 35 °C with continuous oxygenation for 1 h and then washed 3 times with ice-cold sucrose-phosphate buffer (SPB, 0.32 M sucrose, 7.74 mM Na3HPO4, 2.26 mM NaH2PO4, pH 7.4). Afterward tissues were resuspended in 5 ml of SPB, homogenized using a Brinkman Polytron, and centrifuge at 10,000 × g for 10 min at 4 °C. The pellet was resuspended in 5 ml of SPB and centrifuged again at 10,000 × g for 10 min at 4 °C. The resulting pellet was resuspended in 5 ml of SPB and divided into 0.2-ml aliquots in glass test tubes. Aliquots were also saved for measuring protein concentrations. The binding reactions were initiated by adding competing ligands and 0.5 nm [3H]WIN35428 in a total volume of 0.5 ml, then incubated on ice for 2 h and terminated by rapid filtration through Whatman GF/B filters (presoaked in 0.05% polyethyleneimine (PEI)) using a cell harvester (Brandel Instruments, Gaithersburg, MD). The filters were washed twice with 5.0 ml of cold buffer, soaked with scintillation mixture overnight, and measured for radioactivity using a Tri-Carb 2910TR liquid scintillation counter (PerkinElmer Life Sciences) at 50% efficiency. Nonspecific binding measured in the presence of 100 μM cocaine was subtracted from total counts to obtain specific binding. Data were analyzed using GraphPad Prism (San Diego, CA) for non-linear regression to derive Bmax and Kd values. All animal-related protocols were approved by the National Institute on Drug Abuse Intramural Research Program Animal Care and Use Committee.

**DNA subcloning and stable expression cell lines**

The coding sequences of human DAT or human $\sigma_1 R$ cDNA were subcloned into CMV promoter-based mammalian expression vectors: pcDNA3.1(+ (Invitrogen), pCMV-tag5A (Stratagene, La Jolla, CA), or plasmids expressing N-terminal fusion of Myc, HA, GST, or FLAG-2 × Hisa tags that were custom-made in the laboratory and verified by standard DNA sequencing procedures. Plasmids were linearized with PvuI, MluI, or ApaL1 and transfected into HEK293 cells with TransIT LT1 reagent (Mirus Bio, Madison, WI). Cells were then cultured in DMEM (Sigma) with 10% FBS (Invitrogen), penicillin-streptomycin, and 0.5 mg/ml G418 in humidified incubators with 5% CO2 at 37 °C. Expression of DAT and $\sigma_1 R$ in G418-resistant pools or clones was verified by immunoblot using antibodies against DAT, $\sigma_1 R$, or epitope tags. To overcome potential limitations of cell-line specific effects, multiple lines of HEK293 cells were constructed to stably co-express DAT.
and $\sigma_1R$. The following cell lines were used in uptake, binding, or cysteine accessibility assays, including “D-S” (expressing DAT + $\sigma_1R$), “D-SM” (DAT + C-Myc-tagged $\sigma_1R$), “HD-MS” (HA-tagged DAT + N-Myc tagged $\sigma_1R$), and “T316C-MS” (T316C/C306A DAT + N-Myc tagged $\sigma_1R$).

**[^3]HIDA uptake and[^3]HWIN35428 binding in transfected cells**

D-S cells were seeded into PEI-coated 96-well plates and cultured to confluency. After cultured medium was aspirated using an ELx50 plate washer (BioTek, Winooski, VT), cells were washed twice and then incubated in low-Na+ buffer (NaCl 50 mM, 100 mM N-methyl-d-glucamine, 2 mM KCl, 1 mM CaCl2, 1 mM MgCl2, 5 mM glucose, 5 mM HEPES, pH 7.4) for 2 h at 37°C in the absence or presence of $\sigma_1R$ ligands.

For uptake assays, after drug incubation cells were washed twice and then incubated at room temperature for 5 min with 100 $\mu$L of low-Na+ buffer containing 25 nM[^3]HIDA and various concentrations of unlabeled DA and 5 $\muM$ catechol-O-methyl transferase inhibitor Ro 41–0960. Cells were then washed with cold PBSCM using a plate washer and lysed with 0.2 ml of scintillation mixture overnight. Retained radioactivity was determined by a Wallac MicroBeta2 liquid scintillation counter (PerkinElmer Life Sciences). Data were analyzed using the Michaelis-Menten kinetic equation with GraphPad Prism.

For binding assays, after drug incubation D-S cells or HD-MS cells were then washed twice and incubated with low-Na+ buffer containing 7.5 nM[^3]HWIN35428 and various concentrations of unlabeled WIN35428 at 4°C for 1.5 h. Cells were then washed with cold PBSCM using a plate washer, and bound radioactivity was determined as described above. Data were analyzed with GraphPad Prism for non-linear curve-fitting.

**Co-immunoprecipitation and GST pulldown**

HEK293 cells in 60-mm culture dishes were transfected to co-express GST-$\sigma_1R$ and Myc-$\sigma_1R$, DAT, or EAAT2. Two days after transfection, cells were collected and incubated with TNE lysis buffer (1% Triton X-100, NaCl 150 mM, EDTA 1 mM, Tris 10 mM, pH 7.5, and protease inhibitors) at 4°C for 1 h followed by centrifugation at 18,000×g for 20 min at 4°C. Aliquots of the supernatants were saved as lysate input and for protein concentration measurement; the remaining portions were incubated with 40 $\mu$L of 50% slurry of glutathione-conjugated Sepharose beads (prewashed with TNE buffer) on a rotator for 4 h at 4°C. After washing beads three times with TNE lysis buffer, proteins were eluted with SDS sample buffer and separated by SDS-PAGE, transferred to PVDF membranes, and detected by immunoblotting and chemiluminescence methods.

Confluent D-S cells in 60-mm culture dishes were incubated with TNE lysis buffer at 4°C for 1 h and centrifugation at 18,000×g for 20 min at 4°C. Supernatants were incubated with 1 $\mu$g of anti-DAT MAB369 (rat mAb) or normal rat IgG at 4°C for 2 h followed by the addition of 40 $\mu$L of protein A/G beads and incubated for 2 h. Afterward beads were washed three times with TNE lysis buffer. Proteins were eluted and detected as described above.

**BRET assays**

The cDNAs encoding Renilla luciferase (RLuc) or monomeric Venus were fused in-frame to the C terminus of $\sigma_1R$ and N terminus of DAT or EAAT2 in the pcDNA3.1 vector. The BRET assays were performed as described previously (52). Briefly, HEK293T cells were transfected using a constant amount of plasmid DNA but various ratios of plasmids encoding the fusion protein partners. A control corresponding to mock-transfected cells was included in order to subtract raw basal luminescence and fluorescence from the data. Expression of monomeric Venus fusion proteins was estimated by measuring fluorescence at 535 nm after excitation at 485 nm in parallel, BRET was measured as the fluorescence of the cells after incubation with 5 $\muM$ coelenterazine-h. In parallel, BRET was measured as the fluorescence of the cells at 535 nm at the same time points using a Mithras LB940 reader (Berthold Technologies, Bad Wildbad, Germany).

**DAT cysteine accessibility assays and cell-surface biotinylation**

D-SM cells or T316C-MS cells were seeded into PEI-coated 6-well or 12-well plates and cultured to confluency. Cells were washed 3 time with PBSCM + 10 mM glucose (PBSCMG) and then incubated with $\sigma_1R$ ligands in PBSCMG at 37°C for 1 h. Afterward cells were washed 3 times with cold PBSCM, then incubated with 0.5 mg/ml maleimide-PEG2-biotin in the presence or absence of cocaine for 45 min at 4°C with gentle shaking. The labeling reaction was quenched with PBSCM containing 2 mM DTT or 50 mM cysteine for 15 min at 4°C. Cells were then washed, harvested, and lysed in TNE lysis buffer for 1 h at 4°C followed by a 20-min centrifugation at 18,000×g. Aliquots of supernatants were saved for protein concentration measurement. Remaining supernatants were incubated with 60 $\mu$L of NeutrAvidin-agarose beads overnight at 4°C. After washing beads three times with TNE buffer, biotinylated proteins were eluted with SDS sample buffer, separated by SDS-PAGE, transferred to PVDF membranes, and probed with MAB369 or rabbit antisera for DAT. Chemiluminescent signals were captured with a Multilmage III device (Alpha Innotech, San Leandro, CA) as digital TIFF images without pixel saturation. Mean densities of DAT bands were quantified using the NIH ImageJ software and normalized to percent of vehicle. DAT or $\sigma_1R$ expression levels on the cell surface were measured by procedures similar to those outlined above, except that cells were incubated with 0.5 mg/ml sulfo-NHS-SS-biotin or sulfo-NHS-biotin in PBSCM at 4°C for 45 min followed by quenching with 100 mM glycine.

**Analysis of $\sigma_1R$ multimeric states by PFO-PAGE**

Confluent FH-$\sigma_1R$ cells in 12-well plates were incubated with $\sigma_1R$ ligands at 37°C for 1 h in culture medium. Cells were then washed with cold PBSCM, harvested, and incubated with lysis buffer (0.1% GDN, NaCl 150 mM, EDTA 1 mM, Tris 10 mM, pH 7.5, and protease inhibitors) for 1 h at 4°C. After lysisates were centrifuged at 20,000×g for 20 min, supernatants were mixed with an equal volume of 2× PFO sample buffer (8% PFO, 40% glycerol, bromphenol blue 0.005%, Tris 100 mM, pH 7.5) to a final concentration of 4% PFO and heated at 50°C for 10 min.
$\sigma$, $R$ modulates DAT conformation and cocaine binding

Samples were run in 5–15% polyacrylamide Tris-glycine gels (running buffer: 0.1% PFO, 25 mm Tris, 192 mm glycine, pH 8.3). Proteins were transferred to PVDF membranes and im munoblotted with rat monoclonal anti-FLAG L5 antibodies. Because commercial protein markers containing SDS were not suitable in this system, the following proteins were mixed with PFO sample buffer and used as molecular standards: egg white lysozyme (14 kDa), ovalbumin (45 kDa), BSA (66 kDa monomer, 132-kDa dimer), and equine apoferritin (443 kDa).

Author contributions—W. C. H. designed, conducted, and analyzed the experiments in Figs. 2–5 and wrote most of the paper. T. H. and J. L. K. designed, performed, and analyzed the experiments Fig 1. H. Y. designed, conducted, and analyzed the experiments in Fig. 3. C. R. M. and F. T. C. provided novel reagents. S. G. A., T. -P. S., and all other authors contributed to designing experiments, reviewing the results, and writing the paper. All approved the final version of the manuscript.

Acknowledgments—The National Institute on Drug Abuse Drug Supply Program kindly provided (-)-cocaine HCl, (±)-pentazocine succinate, and WIN35428. We thank Dr. Linda Werling for providing human $\sigma$, $R$ cDNA and critical reading of this manuscript.

References
1. Amara, S. G., and Kuhar, M. J. (1993) Neurotransmitter transporters: recent progress. Annu. Rev. Neurosci. 16, 73–93
2. Sulzer, D., Cragg, S. J., and Rice, M. E. (2016) Striatal dopamine neurotransmission: regulation of release and uptake. Basal Ganglia 6, 123–148
3. German, C. L., Baladi, M. G., McFadden, L. M., Hanson, G. R., and Fleckenstein, A. E. (2015) Regulation of the dopamine and vesicular monoamine transporters: pharmacological targets and implications for disease. Pharmacol. Rev. 67, 1005–1024
4. Kristensen, A. S., Andersen, J., Jorgensen, T. N., Sorensen, L., Eriksen, J., Loland, C. I., Stromgaard, K., and Gether, U. (2011) SLc6 neurotransmitter transporters: structure, function, and regulation. Pharmacol. Rev. 63, 585–640
5. Vaughan, R. A., and Foster, J. D. (2013) Mechanisms of dopamine transporter regulation in normal and disease states. Trends Pharmacol. Sci. 34, 489–496
6. Bermingham, D. P., and Blakely, R. D. (2016) Kinase-dependent regulation of monoamine neurotransmitter transporters. Pharmacol. Rev. 68, 888–953
7. Wang, K. H., Pennmatsa, A., and Gouaux, E. (2015) Neurotransmitter and psychostimulant recognition by the dopamine transporter. Nature 521, 322–327
8. Martin, W. R., Eades, C. G., Thompson, J. A., Huppler, R. E., and Gilbert, P. E. (1976) The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. J. Pharmacol. Exp. Ther. 197, 517–532
9. Hanner, M., Moehbius, F. F., Flandorfer, A., Knaus, H. G., Stiessnigg, J., Kempner, E., and Glossmann, H. (1996) Purification, molecular cloning, and expression of the mammalian sigma1-binding site. Proc. Natl. Acad. Sci. U.S.A. 93, 8072–8077
10. Hayashi, T., and Su, T. P. (2001) Regulating ankyrin dynamics: Roles of sigma-1 receptors. Proc. Natl. Acad. Sci. U.S.A. 98, 491–496
11. Aydar, E., Palmer, C. P., Klyachko, V. A., and Jackson, M. B. (2002) The sigma receptor as a ligand-regulated auxiliary potassium channel subunit. Neuron 34, 399–410
12. Kourrich, S., Hayashi, T., Chuang, J. Y., Tsai, S. Y., Su, T. P., and Bonci, A. (2013) Dynamic interaction between sigma-1 receptor and Kv1.2 shapes neuronal and behavioral responses to cocaine. Cell 152, 236–247
13. Hayashi, T., and Su, T. P. (2007) Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca$^{2+}$ signaling and cell survival. Cell 131, 596–610
14. Navarro, G., Moreno, E., Aymerich, M., Marcellino, D., McCormick, P. I., Mallol, J., Cortés, A., Casadó, V., Canela, E. I., Ortiz, J., Fuxe, K., Lluís, C., Ferré, S., and Franco, R. (2010) Direct involvement of sigma-1 receptors in the dopamine D1 receptor-mediated effects of cocaine. Proc. Natl. Acad. Sci. U.S.A. 107, 18676–18681
15. Navarro, G., Moreno, E., Bonaventura, J., Brugarolas, M., Farré, D., Aguina, D., Mallol, J., Cortés, A., Casadó, V., Lluís, C., Ferré, S., Franco, R., Canela, E., and McCormick, P. I. (2013) Cocaine inhibits dopamine D2 receptor signaling via sigma-1-D2 receptor heteromers. PLoS ONE 8, e61245
16. Kim, F. J., Kovalyshyn, I., Burgman, M., Neilan, C., Chien, C. C., and Pastersik, G. W. (2010) Sigma 1 receptor modulation of G-protein-coupled receptor signaling: potentiation of opioid transduction independent from receptor binding. Mol. Pharmacol. 77, 695–703
17. Schmidt, H. R., Zheng, S., Gurpinar, E., Koehl, A., Manglik, A., and Kruse, A. C. (2016) Crystal structure of the human sigma1 receptor. Nature 532, 527–530
18. Walker, J. M., Bowen, W. D., Walker, F. O., Matsumoto, R. R., De Costa, B., and Rice, K. C. (1990) Sigma receptors: biology and function. Pharmacol. Rev. 42, 355–402
19. Gonzalez-Alvear, G. M., and Werling, L. L. (1994) Regulation of $[^3]H$-dopamine release from rat striatal slices by sigma receptor ligands. J. Pharmacol. Exp. Ther. 271, 212–219
20. Narayanan, S., Mesangue, C., Poupaert, J. H., and McCurdy, C. R. (2011) Sigma receptors and cocaine abuse. Curr. Top. Med. Chem. 11, 1128–1150
21. Robson, M. J., Noorbakhsh, B., Seminero, M. J., and Matsumoto, R. R. (2012) Sigma-1 receptors: potential targets for the treatment of substance abuse. Curr. Pharm. Des. 18, 902–919
22. Hiranita, T., Soto, P. L., Tanda, G., and Katz, J. L. (2010) Reinforcing effects of sigma-receptor agonists in rats trained to self-administer cocaine. J. Pharmacol. Exp. Ther. 332, 515–524
23. Hiranita, T., Moreu, M., Soto, P. L., Tanda, G., and Katz, J. L. (2013) Self-administration of cocaine induces dopamine-independent self-administration of sigma agonists. Neuropsychopharmacology 38, 605–615
24. Katz, J. L., Hong, W. C., Hiranita, T., and Su, T. P. (2016) A role for sigma receptors in stimulant self-administration and addiction. Behav. Pharmacol. 27, 100–115
25. Kourrich, S., Su, T. P., Fujimoto, M., and Bonci, A. (2012) The sigma-1 receptor: roles in neuronal plasticity and disease. Trends Neurosci. 35, 762–771
26. Hong, W. C., and Amara, S. G. (2010) Membrane cholesterol modulates the outward facing conformation of the dopamine transporter and alters cocaine binding. J. Biol. Chem. 285, 32616–32626
27. James, M. L., Shen, B., Zavalaeta, C. L., Nielsen, C. H., Mesangue, C., Vuppala, P. K., Chan, C., Avery, B. A., Fishback, J. A., Matsumoto, R. R., Gambhir, S. S., McCurdy, C. R., and Chin, F. T. (2012) New positron emission tomography (PET) radioligand for imaging sigma-1 receptors in living subjects. J. Med. Chem. 55, 8272–8282
28. Katz, J. L., Hiranita, T., Kopajtic, T. A., Rice, K. C., Mesangue, C., Narayanan, S., Abdelazeem, A. H., and McCurdy, C. R. (2016) Blockade of cocaine or sigma or cocaine receptor agonist self-administration by subtype-selective sigma receptor antagonists. J. Pharmacol. Exp. Ther. 358, 109–124
29. Reith, M. E., Berfield, J. L., Wang, L. C., Ferrer, J. V., and Javitch, J. A. (2001) The uptake inhibitors cocaine and benzotropine differentially alter the conformation of the human dopamine transporter. J. Biol. Chem. 276, 29012–29018
30. Pennmatsa, A., Wang, K. H., and Gouaux, E. (2013) X-ray structure of dopamine transporter elucidates antidepressant mechanism. Nature 503, 85–90
31. Palmer, C. P., Mahen, R., Schnell, E., Djamgoz, M. B., and Aydar, E. (2007) Sigma-1 receptors bind cholesterol and remodel lipid rafts in breast cancer cell lines. Cancer Res. 67, 11166–11175
32. Fontanilla, D., Hajipour, A. R., Pal, A., Chu, U. B., Arbabian, M., and Ruoho, A. E. (2008) Probing the steroid binding domain-like I (SBDDL) of
the sigma-1 receptor binding site using N-substituted photoaffinity labels. Biochemistry 47, 7205–7217
33. Pal, A., Chu, U. B., Ramachandran, S., Grawoig, D., Guo, L. W., Hajipour, A. R., and Ruoho, A. E. (2008) Juxtaposition of the steroid binding domain-like I and II regions constitutes a ligand binding site in the sigma-1 receptor. J. Biol. Chem. 283, 19646–19656
34. Hastrup, H., Karlin, A., and Javitch, J. A. (2001) Symmetrical dimer of the human dopamine transporter revealed by cross-linking Cys-306 at the extracellular end of the sixth transmembrane segment. Proc. Natl. Acad. Sci. U.S.A. 98, 10055–10060
35. Sitte, H. H., and Freissmuth, M. (2003) Oligomer formation by Na+-Cl-coupled neurotransmitter transporters. Eur. J. Pharmacol. 479, 229–236
36. Sorkina, T., Doolen, S., Galperin, E., Zahniser, N. R., and Sorkin, A. (2003) Oligomerization of dopamine transporters visualized in living cells by fluorescence resonance energy transfer microscopy. J. Biol. Chem. 278, 28274–28283
37. Torres, G. E., Carneiro, A., Seamans, K., Fiorentini, C., Sweeney, A., Yao, W. D., and Caron, M. G. (2003) Oligomerization and trafficking of the human dopamine transporter: mutational analysis identifies critical domains important for the functional expression of the transporter. J. Biol. Chem. 278, 2731–2739
38. Zhen, J., Antonio, T., Cheng, S. Y., Ali, S., Jones, K. T., and Reith, M. E. (2015) Dopamine transporter oligomerization: impact of combining protomers with differential cocaine analog binding affinities. J. Neurochem. 133, 167–173
39. Chae, P. S., Rasmussen, S. G., Rana, R. R., Gotfryd, K., Kruse, A. C., Manglik, A., Cho, K. H., Nurva, S., Gether, U., Guan, L., Loland, C. J., Byrne, B., Kobilka, B. K., and Gellman, S. H. (2012) A new class of amphiphiles bearing rigid hydrophobic groups for solubilization and stabilization of membrane proteins. Chemistry 18, 9485–9490
40. Ramjeesingh, M., Huan, L. J., Garami, E., and Bear, C. E. (1999) Novel method for evaluation of the oligomeric structure of membrane proteins. Biochem. J. 342, 119–123
41. Kedey, N., Szabo, T., Lile, J. D., Treanor, J. J., Olah, Z., Iadarola, M. J., and Blumberg, P. M. (2001) Analysis of the native quaternary structure of vanilloid receptor 1. J. Biol. Chem. 276, 28613–28619
42. Penna, A., Demuro, A., Yeromin, A. V., Zhang, S. L., Safrina, O., Parker, J., and Cahalan, M. D. (2008) The CRAC channel consists of a tetramer formed by Stim-induced dimerization of Orai dimers. Nature 456, 116–120
43. Xu, J., Liu, Y., Yang, Y., Bates, S., and Zhang, J. T. (2004) Characterization of oligomeric human half-ABC transporter ATP-binding cassette G2. J. Biol. Chem. 279, 19781–19789
44. McCann, D. I., and Su, T. P. (1991) Solubilization and characterization of haloperidol-sensitive (+)-[3H]SKF-10,047 binding sites (sigma sites) from rat liver membranes. J. Pharmacol. Exp. Ther. 257, 547–554
45. Kavanaugh, M. P., Tester, B. C., Scherz, M. W., Keana, J. F., and Weber, E. (1988) Identification of the binding subunit of the sigma-type opiate receptor by photoaffinity labeling with 1-(4-azido-2-methyl[6-3H]phenyl)-3-(2-methyl[4,6-3H]phenyl)guanidine. Proc. Natl. Acad. Sci. U.S.A. 85, 2844–2848
46. Schuster, D. I., Arnold, F. J., and Murphy, R. B. (1995) Purification, pharmacological characterization, and photoaffinity labeling of sigma receptors from rat and bovine brain. Brain Res. 670, 14–28
47. Mishra, A. K., Marlyutov, T., Singh, D. R., Biener, G., Yang, J., Oliver, J. A., Ruoho, A., and Raicu, V. (2015) The sigma-1 receptors are present in monomeric and oligomeric forms in living cells in the presence and absence of ligands. Biochem. J. 466, 263–271
48. Gromek, K. A., Suchy, F. P., Meddaugh, H. R., Wrobel, R. L., LaPonte, L. M., Chu, U. B., Primm, J. G., Ruoho, A. E., Senes, A., and Fox, B. G. (2014) The oligomeric states of the purified sigma-1 receptor are stabilized by ligands. J. Biol. Chem. 289, 20333–20344
49. Carnally, S. M., Johannessen, M., Henderson, R. M., Jackson, M. B., and Edwards, J. M. (2010) Demonstration of a direct interaction between sigma-1 receptors and acid-sensing ion channels. Biophys. J. 98, 1182–1191
50. Balasuriya, D., Stewart, A. P., Crottès, D., Borgese, F., Soriani, O., and Edwardsen, J. M. (2012) The sigma-1 receptor binds to the Nav1.5 voltage-gated Na+ channel with 4-fold symmetry. J. Biol. Chem. 287, 37021–37029
51. Hirainata, T., Soto, P. L., Newman, A. H., and Katz, J. L. (2009) Assessment of reinforcing effects of benztpine analogs and their effects on cocaine self-administration in rats: comparisons with monoamine uptake inhibitors. J. Pharmacol. Exp. Ther. 329, 677–686
52. Sohy, D., Yano, H., de Nadai, P., Urizar, E., Guillabert, A., Javitch, J. A., Parmentier, M., and Springael, J. Y. (2009) Hetero-oligomerization of CCR2, CCR5, and CXCR4 and the protein effects of “selective” antagonists. J. Biol. Chem. 284, 31270–31279