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Characterization of the neurochemical and behavioral effects of solriamfetol (JZP-110), a selective dopamine and norepinephrine reuptake inhibitor*

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Nonstandard Abbreviations

5-HT Serotonin

DA Dopamine

DAT Dopamine transporter

DMSO Dimethyl sulfoxide

ES Excessive sleepiness

FDA United States Food and Drug Administration

IC₅₀ Inhibitor concentration resulting in half inhibition

K_i Inhibitory constant

MWT Maintenance of Wakefulness Test

NE Norepinephrine

NET Norepinephrine transporter

OSA Obstructive sleep apnea

SERT Serotonin transporter

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Abstract

Excessive sleepiness (ES) is associated with several sleep disorders, including narcolepsy and obstructive sleep apnea (OSA). A role for monoaminergic systems in treating these conditions is highlighted by the clinical use of United States Food and Drug Administration–approved drugs that act on these systems, such as dextroamphetamine, methylphenidate, modafinil, and armodafinil. Solriamfetol (JZP-110) is a wake-promoting agent that is currently being evaluated to treat ES in patients with narcolepsy or OSA. Clinical and preclinical data suggest that the wake-promoting effects of solriamfetol differ from medications such as modafinil and amphetamine. The goal of the current studies was to characterize the mechanism of action of solriamfetol at monoamine transporters using *in vitro* and *in vivo* assays. Results indicate that solriamfetol has dual reuptake activity at dopamine (DA; $IC_{50}=2.9\ \mu\text{M}$) and norepinephrine (NE; $IC_{50}=4.4\ \mu\text{M}$) transporters, and this activity is associated *in vivo* with increased extracellular concentration of DA and NE levels as measured by microdialysis. Solriamfetol has negligible functional activity at the serotonin transporter ($IC_{50}>100\ \mu\text{M}$). Moreover, the wake-promoting effects of solriamfetol are likely due to activity at DA and NE transporters rather than other neurotransmitter systems, such as histamine or orexin. The dual activity of solriamfetol at DA and NE transporters and the lack of significant monoamine-releasing properties of solriamfetol might explain the differences in the *in vivo* effects of solriamfetol compared with modafinil or amphetamine. Taken together, these data suggest that solriamfetol may offer an important advancement in the treatment of ES in patients with narcolepsy or OSA.

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Introduction

Excessive sleepiness (ES) is present in a wide range of sleep disorders, including narcolepsy and obstructive sleep apnea (OSA). In narcolepsy, ES is the clinical hallmark symptom, and is the symptom reported as having the most significant impact on patients' lives (Morgenthaler et al., 2007; United States Department of Health and Human Services, Food and Drug Administration [FDA] Narcolepsy Public Meeting, 2013). Moreover, the American Academy of Sleep Medicine recommends that a reduction in ES is one of the most critical aspects in the management of narcolepsy (Krahn et al., 2015). ES in OSA is a prominent symptom and may not resolve despite effective therapy with positive airway pressure (Weaver and Chasens, 2007; Veasey et al., 2006; Pepin et al., 2009; Gasa et al., 2013; Chapman et al., 2016). In patients with narcolepsy or OSA, ES can be a debilitating condition and often has a substantial impact on patients' quality of life (Ozaki et al., 2008; Aldrich, 1989; George, 2007).

Monoaminergic systems [serotonin (5-HT), norepinephrine (NE) and dopamine (DA)] play a well-recognized role in a variety of physiologic functions, including sleep-wake regulation (Jouvet, 1972; Steriade and McCarley, 1990; Jones, 2000; Siegel, 2000; Wisor et al., 2001). Although the underlying pathophysiology of ES may differ among sleep disorders (for review, see Slater and Steier, 2012), several lines of evidence associate ES, in part, with dysregulation of DA systems (Dauvilliers et al., 2015; Burgess et al., 2010; Zhu et al., 2007). First, in preclinical studies, drugs with actions on DA systems such as stimulants (e.g., amphetamine) and DA reuptake inhibitors (e.g., modafinil) dose-dependently increase wakefulness in wild-type mice and in animal models of narcolepsy (Nishino et al., 1998; Wisor et al., 2001; Mignot et al., 1994). Second, the *in vitro* affinity of DA reuptake inhibitors for the DA transporter (DAT)

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significantly correlates with the *in vivo* potency of those reuptake inhibitors to induce wake-promoting effects (Nishino et al., 1998). Third, a link between DA function and ES is suggested by the clinical use of currently available therapies with actions on these systems, such as dextroamphetamine (Dexedrine Prescribing Information, 2017), methylphenidate (Ritalin Prescribing Information, 2017), modafinil (Provigil Prescribing Information, 2007), and armodafinil (Nuvigil Prescribing Information, 2015) in patients with narcolepsy or OSA. However, the use of each of these medications is associated with limitations such as limited tolerability, suboptimal response, and abuse potential (Bastuji and Jouvet, 1988; Dauvilliers et al., 2002; Mignot and Nishino, 2005; Thorpy and Dauvilliers, 2015). These findings highlight the need for additional effective and tolerable therapies to improve wakefulness and to treat ES associated with narcolepsy or OSA.

Solriamfetol, (*R*)-2-amino-3-phenylpropylcarbamate hydrochloride, formerly known as JZP-110, (Figure 1) is a wake-promoting agent that is being evaluated as a potential treatment for ES in patients with narcolepsy or OSA. Collectively, clinical and preclinical data suggest that the wake-promoting effects of solriamfetol differ from currently approved medications such as modafinil and amphetamine. Although head-to-head comparisons of solriamfetol and other medications have not been conducted, the clinical effects of solriamfetol on wakefulness in narcolepsy may differ compared with the effects observed in previous studies of modafinil and armodafinil (Harsh et al., 2006; Schwartz et al., 2003; Ruoff et al., 2016; Ruoff et al., 2017). For instance, a post-hoc analysis of a Phase 2b clinical trial of solriamfetol in patients with narcolepsy resulted in maximal differences between drug and placebo groups of 7.8 minutes on a 40-minute Maintenance of Wakefulness Test (MWT) that was censored to 20-minutes to

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facilitate comparisons with data from other studies (Ruoff et al., 2017). In contrast, previous studies of modafinil and armodafinil showed maximal differences between drug and placebo groups ranging from 2.3 to 4.5 minutes using a 20-minute MWT (US Modafinil in Narcolepsy Multicenter Study Group 1998, 2000; Harsh et al., 2006). In a preclinical study, solriamfetol induced wakefulness in a manner that differed from *d*-amphetamine in mice (Hasan et al., 2009). In contrast to *d*-amphetamine (6 mg/kg), the effects of an equipotent wake-promoting dose of solriamfetol (150 mg/kg) were not accompanied by stereotyped behavior or pronounced locomotor activity, and furthermore, this dose of solriamfetol did not produce rebound hypersomnia (i.e., increase in sleep above baseline after pharmacologic promotion of wakefulness; Hasan et al., 2009). In order to better understand differences in the therapeutic effects between solriamfetol and other wake-promoting drugs, the current studies were undertaken to characterize the mechanism of action of solriamfetol compared with stimulants and DA reuptake inhibitors.

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Methods

In Vitro Transporter Assays

Binding and functional assays were carried out in transfected cells expressing cloned human monoamine transporters using methods described previously (Janowsky et al., 2001; Eshleman et al., 1999, 2013). In brief, human embryonic kidney (HEK293) cells expressing recombinant DNA for the human DAT (hDAT), 5-HT transporter (hSERT), or NE transporter (hNET) were grown to 80% confluence on 150-mm diameter tissue culture dishes. To prepare cell membranes, medium was removed and cells were washed with 10 mL of calcium- and magnesium-free phosphate-buffered saline (PBS). Lysis buffer (10 mL; 2 mM HEPES with 1 mM EDTA) was added. After 10 min, cells were scraped from plates and centrifuged at 30,000g for 20 min. The supernatant fluid was removed and the pellet was resuspended in 12-32 mL of 0.32 M sucrose. Each assay tube contained 50 μ L of membrane preparation, 25 μ L of test compound (concentration range 21.6 nM-100 μ M for hDAT and hSERT and 21.6 nM-10 μ M for hNET), compound used to define non-specific binding or buffer, 25 μ L of [125 I]RTI-55 (40-80 pm final concentration; specific activity 2200Ci/mmol), and additional buffer sufficient to bring up the final volume to 250 μ L. Membranes were preincubated with test compounds for 10 min prior to the addition of the [125 I]RTI-55 followed by incubation at 25°C for 90 min. Binding was terminated by filtration over grade C glass- microfiber (GF/C) filters using a 96-well cell harvester, filters were washed with ice-cold saline, and scintillation fluid was added. Specific binding was defined as the difference in binding observed in the presence and absence of 5 μ M mazindol (hDAT and hNET) or 5 μ M imipramine (hSERT). Two or three independent experiments were conducted with duplicate (binding) or triplicate (reuptake and release) determinations. GraphPad Prism (GraphPad Software, Inc., La Jolla, CA) was used to convert

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IC₅₀ values to K_{0.5} values using the bimolecular Cheng-Prusoff relationship (Cheng and Prusoff, 1973). Additionally, reuptake and release of [³H]DA, [³H]NE, and [³H]5-HT in HEK293 cells were performed as described above (Janowsky et al., 2001; Eshleman et al., 1999, 2013). For reuptake, the concentration of solriamfetol tested was 31.6 nM-100 μM for hSERT and hNET and 31.6 nM-10 μM for hDAT. For release, the concentration of solriamfetol tested was 1 nM-10 μM for hSERT and hNET and 1nM-100 μM for hDAT.

Binding assays for monoamine transporters were also carried out in rat brain membranes using methods described previously (Walker and Mailman, 1996). In brief, tissue was homogenized in 50 mM Tris-HCl, pH 7.4 buffer, centrifuged (23,500g, 4°C, 10 minutes) and diluted in assay buffer, prior to protein content measurement. For [³H]WIN35428 binding, 250 μg of homogenized rat striatum was incubated (buffer: 10 mM Na₂HPO₄, 320 mM sucrose, pH 7.45) with test compound for 15 minutes at 21°C in the presence and absence of mazindol (1 μM). Reuptake and release assays for DAT, NET, and SERT were carried out in rat brain synaptosomes using published methods. For reuptake inhibition assays, striatum was used for DA reuptake assays, and hypothalamus for NE reuptake studies using methods described previously (Heffner and Seiden, 1980; Mottola et al., 1992). For release assays, rat striatum was used for DA release and rat frontal cortex for 5-HT and NE release using methods described previously (Mottola et al., 1992). Each experimental point was conducted with triplicate determinations.

***In Vitro* Receptor Assays**

Receptor binding studies were conducted with several reference ligands (chosen for their affinity and selectivity for the particular receptor in each assay) in membrane extracts from tissue

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expressing a receptor of interest, or from cell lines expressing the transfected receptor. Control wells (blank) contained cold reference compounds in sufficient concentration to block the binding of the radioligand. Generally, frozen membranes were thawed and briefly homogenized prior to dilution to an appropriate protein concentration optimized for specific and non-specific binding. Radioligand was prepared by dilution in assay buffer to a suitable concentration, then incubated with a 10% dimethyl sulfoxide (DMSO) control (final concentration 1% DMSO), solriamfetol (10^{-9} M to 10^{-5} M, and in the case of adrenergic receptors $h\alpha_{2A}$, $h\alpha_{2B}$, and $h\alpha_{2C}$ up to 10^{-4} M), or competing cold compound, plus membrane preparations from cells or tissue expressing the receptor of interest. Binding to D₁-like and D₂-like receptors used methods as described previously (Lewis et al., 1998; Mottola et al., 2002). For measurement of 5-HT_{1A} functional activity, agonist activation was determined by comparison with 5-HT (10 μ M) as a control (100%). Antagonism was determined by inhibition of GTP γ S binding by compound in the presence of a sub-maximal activation concentration of 5-HT (0.1 μ M). Membrane-bound activity was collected by filtration either through a Packard Filtermate 196 Harvester (Hewlett Packard, San Diego, CA) onto UniFilter plates (cell membranes; GE Healthcare Life Sciences, Pittsburgh, PA) or by Brandel tissue harvester (Brandel, Inc., Gaithersburg, MD) onto grade GF/B filters, and by subsequent washing with ice-cold buffer (3 x 4 mL) or by scintillation proximity assay-based detection methods. Membranes were allowed to dry before adding scintillation fluid and counting in a TopCount scintillation counter (PerkinElmer Life and Analytical Sciences, Waltham, MA). Percentage specific bound and competition binding curves were calculated using S-Plus software (Statistical Sciences, Seattle, WA).

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Binding to the cloned human histamine H₃ receptor was carried out in transfected SK-N-MC cells, using methods described previously (Lovenberg et al., 2000). Briefly, cell pellets from SK-N-MC cells expressing the human H₃ receptor were homogenized in 50 mM Tris-HCl/5 mM EDTA and recentrifuged at 30,000g for 30 minutes. Then, pellets were homogenized in 50 mM Tris/5 mM EDTA (pH 7.4). Membranes were incubated with 0.8 nM N-[³H]- α -methylhistamine plus/minus test compounds for 45 minutes at 25°C and harvested by rapid filtration over GF/C filters (pretreated with 0.3% polyethylenimine) followed by four washes with buffer.

Nonspecific binding was defined in the presence of 100 μ M histamine.

Orexin receptor-2 functional assays were performed as described previously (McAtee et al., 2004). Briefly, PFSK-1 cells (human neuroectodermal cell line expressing human orexin receptor-2) were stimulated with agonists of orexin receptor-2 to increase intracellular Ca⁺⁺, which was measured with the FLIPR[®] instrument (Molecular Devices, LLC, Sunnyvale, CA). On day 1, the PFSK-1 cells were plated onto 96-well Packard View Plates in RPMI 1640 (Thermo Fisher Scientific, Boston, MA) supplemented with 10% fetal bovine serum and penicillin/streptomycin, then allowed to grow overnight at 37°C. On day 2, the medium was removed and replaced with Dulbecco's modified Eagle's medium nutrient mixture F-12 (Thermo Fisher Scientific, Boston, MA) containing Fluo-3 AM (a Ca⁺⁺-sensing fluorescent dye) and F-127 detergent. After allowing the cells to load the fluorescent dye for 1 hour at room temperature, the FLIPR instrument was used to apply various doses of solriamfetol and to follow any changes in intracellular Ca⁺⁺ for 2 minutes. After an additional 10-minute incubation at room temperature, the same cell plate was assayed for the Ca⁺⁺ response to 100 nM orexin-A in the same manner.

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Solriamfetol was also tested at a concentration of 1 μM in a battery of additional assays for inhibition of radioligand binding by CEREP (Celle L'Evescault, France) that included human A_1 , A_{2A} , and A_3 adenosine receptors, α_1 - and α_2 -adrenergic receptors, human β_1 -adrenergic receptor, human AT_1 angiotensin receptor, benzodiazepine receptor, human bradykinin receptor, human CCK_1 cholecystokinin receptor, human D_1 and D_2 dopamine receptors, human endothelin receptor type A, $GABA_A$ receptor, human galactose transporter, human CXC chemokine receptors, human C-C chemokine receptor type 1, H1 and H2 histamine receptors, human MC_4 melanocortin receptor, MT1 melatonin receptor, human M_1 , M_2 , and M_3 muscarinic acetylcholine receptors, human NK_1 and NK_3 neurokinin receptors, human Y_1 and Y_2 neuropeptide receptors, human NTS_1 neurotensin receptor, human μ -, δ -, and κ -opioid receptors and opioid-like receptor, human 5-HT_{1A} , 5-HT_{1B} , 5-HT_{2A} , 5-HT_3 , 5-HT_{5A} , 5-HT_6 , and 5-HT_7 serotonin receptors, somatostatin receptor, human vasoactive intestinal peptide receptor, human vasopressin receptor, Ca^{2+} channel, K^+_v channel, SK^+_{Ca} channel, Na^+ channel, and Cl^- channel. Solriamfetol also was tested at a concentration of either 1 or 10 μM in a panel of more than 100 kinases (KinaseProfiler screening service; Upstate Biotechnology, Inc., Charlottesville, VA).

Ex Vivo Autoradiography

α_2 -adrenergic receptor density was assessed via [^3H]RS79948-197 and [^3H]rauwolscine binding to brain areas showing high receptor density (septum and caudate putamen) using methods reported previously (Schotte et al., 1996). Briefly, rats were treated by subcutaneous administration of vehicle or solriamfetol at a dose of 40 mg/kg (3 animals per treatment). The animals were decapitated 1 h after administration. Brains were immediately removed from the skull and rapidly frozen in 2-methylbutane on dry ice and stored at -40°C . Twenty μm -thick

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sections were cut using a Leica CM 3050 cryostat-microtome (van Hopplynus, Belgium), and thaw mounted on microscope slides (SuperFrost Plus Slides, LaboNord, France). The sections were then kept at -20°C until use. After thawing, sections were dried under a stream of cold air. The sections were not washed prior to incubation to avoid dissociation of the drug receptor complex. Brain sections were incubated for 10 min in Tris-HCl buffer (50 mM, pH 7.4) containing 3 nM [³H]RS79948-197 or in Na-K-Phosphate buffer (50 mM, pH 7.7) containing 0.8 nM [³H]rauwolscine. Nonspecific binding was determined by slides containing 10 μM rauwolscine (for [³H]RS79948-197 binding) or by slides containing 10 μM phentolamine (for [³H]rauwolscine binding). After the incubation, the excess of radioactivity was washed off in consecutive baths of ice-cold buffer, followed by a quick rinse in ice-cold water. The sections were then dried under a stream of cold air. Brain slices incubated with tritiated ligands were loaded in the β-imager (Biospace, Paris) for 1 h. Acquired images were quantified using the Beta vision program (Biospace, Paris). Specific binding was given as the difference between total binding and nonspecific binding measured in adjacent sections.

Animals

All animals were maintained under controlled environmental conditions on a 12-h light-dark cycle with standard laboratory chow and water available *ad libitum* (with the exception of drug discrimination experiments described below). For *in vitro* binding and functional assays using rat brain synaptosomes, male Sprague-Dawley rats weighing 200 to 400 g were purchased from Charles River Laboratories (Raleigh, NC). For *ex vivo* autoradiography, male Wistar rats weighing 200 g were purchased from Charles River Laboratories (Germany). For microdialysis experiments, male Sprague-Dawley rats (Harlan, Horst, The Netherlands) weighing 300 to 400 g were individually housed in full-view Plexiglass containers (25 x 33 x 18 cm) located in a sound-

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attenuated room. The animals were allowed to acclimate for at least 7 days prior to surgery. For locomotor experiments, male Swiss-Webster mice were obtained from Harlan (Indianapolis, IN) and were group housed. For drug discrimination experiments, male Sprague-Dawley rats were obtained from Harlan (Indianapolis, IN) and were housed individually. Body weights were maintained at 320 to 350 g by limiting food to 15 g/day; water was continuously available in the home cage. The Institutional Animal Care and Use Committees approved all animal procedures.

***In Vivo* Microdialysis**

Thirty-six rats were surgically implanted with two guide cannulas (CMA Microdialysis AB, Kista, Sweden): one into the striatum and the other into the prefrontal cortex using methods described previously (Gregory et al., 2013). Briefly, rats were anesthetized with isoflurane and O₂ 30%/N₂O 70% mixture inhalation anesthesia and were mounted in a stereotactic apparatus while situated on an isothermic heating blanket that maintained their core body temperature at 37°C. Two small holes were drilled through the skull at the following coordinates taken from Paxinos and Watson (1998): incisor bar set at -3.5 mm, +2.75 mm anterior to and 0.75 mm lateral to bregma for the prefrontal cortex, and +0.5 mm anterior to and 2.5 mm lateral to bregma for the striatum. Five more holes were made in the skull, two adjacent to the cannulae and three in the contralateral parietal plate. Five self-tapping gold-plated anchor screws were screwed in these holes and two guide cannulae (CMA/12) with inserted dummy probes were lowered into the brain. The cannulae were lowered through the surface of the brain (tips ventral at 0.75 mm for the prefrontal cortex and at 3.25 mm for the striatum) and glued to the screws using 3M dental cement. Screws and cannulae were then embedded within dental cement to smooth the headset. After surgery, the animals were housed individually and allowed to recover for at least

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10 days prior to experimentation. After the experiment, all animals were sacrificed for histologic verification of probe placement: an animal was excluded from the analysis if other brain structures were affected or if lesions were found with more than 0.5 mm divergence from the target coordinates in two or more planes.

One day prior to testing, rats were transferred to the experimental room and inserted with microdialysis probes. The next day, dialysates were collected in three consecutive 50-minute baseline samples, followed by eight consecutive 50-minute post-administration samples of vehicle (saline, 0.9% NaCl) or solriamfetol [10 and 30 mg/kg, administered s.c.] in separate groups of rats (n = 12/condition). Microdialysates were analyzed by high-performance liquid chromatography with a Waters 2465 electrochemical detector and 0.7-mm capillary cell with glassy carbon electrode. Data are presented as the average percent change in neurotransmitter concentration of a certain post-administration sample compared with the individual animal's average baseline value (i.e., the mean for that animal over the three baseline samples). A total of three baseline samples and eight consecutive post-administration samples were analyzed. The average value and corresponding SEM were then calculated for each 50-minute sample.

Drug Discrimination

Six rats were trained to discriminate cocaine (10 mg/kg) from saline using a two-lever choice task. Food was available as a reinforcer under a fixed ratio 10 schedule (FR10) when responding occurred on the injection-appropriate lever. All tests occurred in standard, commercially available chambers (Coulbourn Instruments, Holliston, MA), using 45-mg food pellets (Bio-Serv, Flemington, NJ) as reinforcers. Training sessions occurred in a double-alternating fashion,

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and tests were conducted between pairs of identical training sessions (i.e., between either two saline or two cocaine training sessions). Tests occurred only if, in the two preceding training sessions, rats met the criteria of at least 85% of responses on the injection-appropriate (i.e., the “correct”) lever for both the first reinforcer (first fixed ratio) and the total session. Test sessions lasted for 20 min, or until 20 reinforcers had been obtained. Injections (1 mg/kg i.p.) of solriamfetol or its vehicle (0.9% saline), occurred 60 minutes prior to the start of the test session. Drug discrimination data are expressed as the average percentage of the total responses occurring on the cocaine-appropriate lever during the entire response period.

Locomotor Activity

Separate groups of mice (n = 8/condition) were injected with either vehicle (0.9% saline), cocaine (5, 10, 20, or 40 mg/kg; i.p.), or solriamfetol (3, 10, 30, or 100 mg/kg; i.p.) immediately prior to locomotor activity testing. The locomotor study was conducted using 40 digiscan locomotor activity testing chambers (Omnitech Electronics, Inc, Columbus, OH), measuring 40.5 x 40.5 x 30.5 cm, and housed in sets of two within sound-attenuating chambers. A panel of infrared beams (16 beams) and corresponding photodetectors were located in the horizontal direction along the sides of each activity chamber. A 7.5-W incandescent light above each chamber provided dim illumination. Fans provided an 80-dB ambient noise level within the chamber. In all studies, horizontal activity (interruption of photocell beams) was measured for 8 hours within 10-minute periods, beginning at 8:00 AM (2 hours after lights on). Testing was conducted with one mouse per activity chamber. For presentation of locomotor data, the average effect was taken for each animal during the first 30 minutes of the recording period. The

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statistical significance of differences between the mean of vehicle and the mean of each dose of drug was assessed by a Student's *t*-test.

Chemicals, Reagents, and Drug Treatments

Solriamfetol was provided by SK Life Science, Inc., (Fair Lawn, NJ) or Janssen Pharmaceuticals (Raritan, NJ). For *in vitro* binding and functional assays, radioligands were purchased commercially from either NEN Life Sciences (Boston, MA), Amersham Biosciences (Little Chalfont, United Kingdom), or PerkinElmer Life and Analytical Sciences (Waltham, MA), including: [³H]mazindol, [³H]citalopram, [³H]GBR-12909, [¹²⁵I]RTI-55, [³H]DA, [³H]NE, and [³H]5-HT. The following drugs were received as gifts: fluoxetine (Lilly Research Laboratories, Indianapolis, IN) and GBR-12909 (Novo Industries, Bagsvaerd, Denmark). HEPES buffer was purchased from Research Organics, Inc (Cleveland, OH). Other drugs and reagents were of highest available purity and were purchased from commercial sources.

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Results

In Vitro Transporter and Receptor Assays

The first set of binding studies examined the interaction of solriamfetol with monoamine transporters (DAT, NET, and SERT) in transfected cells and rat brain synaptosomes. As indicated in Table 1, solriamfetol binds to DAT and NET. The affinity of solriamfetol for these transporters was lower than that of cocaine in transfected cells. For example, the binding affinity (K_i) of solriamfetol for DAT was 60-fold lower than the binding affinity of cocaine for DAT (K_i values = 14,200 and 236 nM, respectively) and the binding affinity of solriamfetol for NET was 7-fold lower than the binding affinity of cocaine for NET (K_i values = 3,700 and 505 nM, respectively; Table 1). Further, solriamfetol had negligible binding affinity at the SERT compared with cocaine (K_i values = 81,500 and 361 nM, respectively). Studies conducted in rat striatal synaptosomes replicated and extended the finding that solriamfetol interacts with DAT (Table 1). In these studies involving rat brain tissue, solriamfetol had a lower affinity for DAT compared with cocaine. For example, cocaine displaced [3 H]WIN35428 binding with an IC_{50} of 160 nM, whereas solriamfetol displaced [3 H]WIN35428 binding with an IC_{50} of 2600 nM (Table 1).

Monoamine reuptake and release assays were conducted to investigate how solriamfetol functionally interacts with monoamine transporters. In transfected cells, solriamfetol blocked the reuptake of [3 H]DA and [3 H]NE (IC_{50} = 2900 and 4400 nM, respectively; Table 2). However, solriamfetol was a less potent inhibitor compared with cocaine (IC_{50} = 385 and 194 nM, at DAT and NET respectively; Table 2). Further, solriamfetol did not have significant functional activity at blocking the reuptake of [3 H]5-HT, compared with cocaine. Additional studies in rat brain

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synaptosomes confirmed and extended the finding that solriamfetol has relatively low (μM) potency for blocking the reuptake of [^3H]DA and [^3H]NE in brain tissue, compared with reference ligands with high potency for monoamine reuptake inhibition (e.g., GBR-12909, bupropion, desipramine; Table 2). In release assays, solriamfetol had no appreciable effect on the release of preloaded [^3H]DA, [^3H]NE, or [^3H]5-HT in transfected cells (Table 2). In a study with rat brain synaptosomes, the highest concentration of solriamfetol tested ($30\ \mu\text{M}$) resulted in DA and 5-HT release (approximately 70% and 30% of that of the respective reference compounds) and no release of NE (Figure 2).

Results from the receptor binding assays in heterologous transfected cells showed that solriamfetol (10 or $100\ \mu\text{M}$) did not have significant affinity for any receptors except $5\text{-HT}_{1\text{A}}$ and α_2 -adrenergic receptors (Table 3). Subsequent functional assays demonstrated that solriamfetol did not have any functional activity at $5\text{-HT}_{1\text{A}}$ receptors (neither stimulation nor inhibition of 5-HT-induced $\text{GTP}\gamma\text{S}$ binding) even at the maximum concentration ($10\ \mu\text{M}$) tested. Further, a subsequent study evaluated α_2 -adrenergic binding sites by quantitative autoradiography using two different α_2 -adrenergic radioligands [^3H]RS79948-197 or [^3H]rauwolscine. There were no differences in either [^3H]RS79948-197 or [^3H]rauwolscine binding density between solriamfetol- and vehicle-treated rats. Of note, solriamfetol had no significant interactions with histamine H_1 or H_3 receptors (i.e., $K_i > 10\ \mu\text{M}$), did not activate orexin-2 receptors, and did not inhibit the stimulatory effects of orexin-A. No additional significant interactions were observed in evaluations performed at CEREP (see Materials and Methods for the assays included). In the kinase screen, no broad enzyme inhibition or activation was observed (including monoamine

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oxidase A and B), suggesting that solriamfetol is not a non-specific kinase inhibitor (see Materials and Methods for the assays included).

Drug Discrimination

In order to evaluate the mechanism of action of solriamfetol *in vivo*, solriamfetol was tested for its ability to substitute for cocaine in rats trained to discriminate cocaine from saline. In six rats trained to discriminate 10 mg/kg cocaine from saline, solriamfetol occasioned responding on the cocaine-appropriate lever in a dose-dependent manner, with a dose of 100 mg/kg occasioning more than 80% cocaine-appropriate responding (solriamfetol ED₅₀: 37.6 mg/kg [95% CI: 6.63 – 213.41 mg/kg]; Figure 3A). Solriamfetol also dose-dependently decreased the rate of responding such that a 100-mg/kg dose of solriamfetol resulted in a significant decrease in response rate to approximately 40% of control and 1 of 5 rats failed to complete the first fixed-ratio (Figure 3B).

Locomotor Activity

To examine whether solriamfetol shared other behavioral effects with cocaine, the locomotor effects of solriamfetol were compared with cocaine. As shown in Figure 4A, cocaine dose-dependently increased locomotor activity resulting in an inverted U-shaped curve. The maximum average (\pm SEM) effect was 6817 (\pm 545) ambulation counts at 20 mg/kg cocaine compared with 3599 (\pm 178) ambulation counts with vehicle (8 mice/treatment; $P < 0.05$). In contrast to the dose-response curve for cocaine, Figure 4B shows that the effects of solriamfetol on locomotor activity were minimal with decreases observed at the largest dose (100 mg/kg; $P < 0.05$). The maximum average effect was 3802 (\pm 131) ambulation counts at 30 mg/kg solriamfetol compared with 2997 (\pm 166) ambulation counts with vehicle ($P < 0.05$).

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***In Vivo* Microdialysis**

For microdialysis experiments, a maximal dose of 30 mg/kg solriamfetol was selected based on the observation that doses >30 mg/kg solriamfetol decreased rate of responding (albeit a different route of administration [s.c.] was used for the drug discrimination experiment). At a dose of 30 mg/kg, but not 10 mg/kg, solriamfetol increased striatal DA extracellular levels in freely moving conscious rats sampled via *in vivo* brain microdialysis (Figure 5A). Specifically, solriamfetol (30 mg/kg) produced a peak increase of approximately 350% in DA levels approximately 125 minutes after injection, compared with baseline. Solriamfetol-induced increases in DA levels were observed through the remainder of the sampling period (Figure 5A). Approximately 75 minutes after injection, solriamfetol (30 mg/kg) produced a peak increase of approximately 350% in prefrontal cortical NE extracellular levels compared with baseline (Figure 5B). Solriamfetol-induced increase in NE levels returned to baseline around 275 minutes after injection (i.e., sampling period at 450 min; Figure 5B). No effects of solriamfetol (at doses of 10 and 30 mg/kg) on 5-HT levels could be detected in either the striatum or frontal cortex compared with baseline levels (Jazz Pharmaceuticals, data on file).

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Discussion

Collectively, data from the current studies demonstrate that the mechanism of action of solriamfetol includes low potency DA and NE reuptake inhibition via DAT and NET, respectively, without significant activity at any other target studied to date. In rats, a dose of solriamfetol (35 mg/kg; p.o.) produces a plasma C_{max} of 23.1 μ M (Jazz Pharmaceuticals, data on file); brain concentrations are expected to be similar to the plasma concentrations based on the high solubility, high permeability, and low plasma protein binding of solriamfetol. The plasma concentration of 23.1 μ M is higher than both the binding affinity (K_i) for DAT and NET and the IC_{50} for DA or NE reuptake inhibition (see Tables 1 and 2), suggesting that the *in vivo* effects of solriamfetol in rodents are mediated through mechanisms involving DAT and NET. Consistent with this notion, microdialysis experiments showed that a dose of 30 mg/kg (s.c.) solriamfetol increased DA and NE levels in the striatum and prefrontal cortex, respectively. In addition, the finding that solriamfetol generalized to cocaine in rats trained to discriminate cocaine from saline supports the conclusion that solriamfetol has activity at DAT and NET *in vivo* as several studies support a role for DA and NE reuptake in the discriminative stimulus effects of cocaine (Kleven et al., 1990; Baker et al., 1993; Spealman, 1995). Together, these data suggest that the behavioral effects (e.g., wake-promoting effects) of solriamfetol are due to its activity at DAT and NET, rather than other neurotransmitter receptors involved in sleep-wake regulation, such as histamine or orexin receptors (Lin et al., 2011; Dauvilliers et al., 2013; De la Herrán-Arita and García-García, 2013).

Results from the binding and reuptake studies indicate that solriamfetol has negligible binding affinity and functional activity at SERT (Tables 1 and 2). In addition, solriamfetol (at doses of 10

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and 30 mg/kg) did not increase 5-HT extracellular levels in brain as shown in microdialysis experiments. Data from other studies have also demonstrated that solriamfetol does not have a serotonergic mechanism of action *in vivo* based on findings that solriamfetol did not resemble the effects of fluoxetine, a selective serotonin reuptake inhibitor. For instance, 5-hydroxytryptophan (5-HTP) induces head twitching in rodents (Corne et al., 1963), which is presumed to be mediated by the 5-HT_{2A} receptor subtype (Halberstadt et al., 2011; Canal and Morgan, 2012). In contrast to the effects of fluoxetine, which shifted the 5-HTP dose-response curve for head twitches to the left in mice, solriamfetol (30 and 60 mg/kg) did not potentiate 5-HTP-induced head twitches (Jazz Pharmaceuticals, data on file). Together, the *in vitro* and *in vivo* data indicate that solriamfetol does not have activity at SERT or 5-HT receptors.

Several lines of evidence suggest that the mechanism of action of solriamfetol differs from currently available stimulants (e.g., amphetamines) and wake-promoting agents (e.g., modafinil) with regard to activity at monoamine transporters. For instance, and in contrast to the well-known monoamine-releasing effects of amphetamine (see Rothman et al., 2001), solriamfetol is neither a potent nor efficacious monoamine releaser. In rat brain synaptosomes, the highest concentration of solriamfetol tested (30 μ M) resulted in sub-maximal DA and 5-HT release (70% and 30%, respectively) and did not promote release of NE (Figure 2). The partial release that was observed at 30 μ M solriamfetol is not likely to be relevant at therapeutic doses based on the observation that oral daily administration of the highest therapeutic dose of solriamfetol (300 mg) in clinical studies produces a steady state plasma C_{max} of 8.6 μ M (1670 ng/ml) in humans (Jazz Pharmaceuticals, data on file). However, further studies are necessary to elucidate the underlying mechanism(s) related to the partial DA release that was observed at 10 μ M

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solriamfetol (40%) and whether this effect might contribute to the clinical effects of the highest therapeutic dose in humans. Partial DA release that was observed at higher concentrations of solriamfetol is not likely explained by actions of metabolites. Solriamfetol is primarily excreted unchanged in urine with $\leq 1\%$ of the dose recovered as the minor metabolite N-acetyl solriamfetol and an *in vitro* binding study indicated that this metabolite did not display any binding to DAT or NET (Jazz Pharmaceuticals, data on file). Nevertheless, the neurochemical differences regarding the interaction at monoamine transporters between solriamfetol and amphetamine (i.e., reuptake inhibitor versus releaser) might help explain why rebound hypersomnia was observed after administration of amphetamine, but not after administration of an equipotent wake-promoting dose of solriamfetol in mice (Hasan et al., 2009).

The behavioral effects of solriamfetol are similar, but not identical, to the effects of DA reuptake inhibitors and traditional stimulants. For instance, solriamfetol fully substituted for cocaine discriminative stimulus effects, with the largest dose (100 mg/kg) occasioning more than 80% cocaine-appropriate responding, likely based on the overlapping mechanism of action of solriamfetol and cocaine (i.e., activity at DAT/NET). However, cocaine dose-dependently increased locomotor activity, resulting in an inverted U-shaped dose-response curve, whereas the effects of solriamfetol on locomotor activity were minimal and the maximum effect was lower compared with the effects of cocaine. Finally, solriamfetol does not produce conditioned place preference or self-administration, unlike cocaine and other psychostimulants (Jazz Pharmaceuticals, data on file; Baladi et al., 2016; Baladi et al., 2017; Carroll and Lac, 1997; Spyraiki et al., 1982; Stuber et al., 2002). These differences in the behavioral effects of solriamfetol and cocaine might be due to differences in the manner in which extracellular DA

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levels are increased. For instance, the increase of DA levels produced by solriamfetol is lower in magnitude and has a slower onset compared with the effects of cocaine on DA levels (Loland et al., 2012).

Results from several studies make a compelling case that the wake-promoting effects of modafinil are primarily mediated by its interaction at DAT and elevation of DA levels (for review, see Wisor, 2013). First, the wake-promoting effects of modafinil are absent in DAT-knockout mice (Wisor et al., 2001) and are attenuated by DA receptor antagonists in wild-type mice (Qu et al., 2008). Second, modafinil increases extracellular levels of DA in brain (de Saint Hilaire et al., 2001; Wisor et al., 2001; Murillo-Rodriguez et al., 2007). Third, selective DAT ligands (e.g., GBR-12909) substitute for modafinil in rats trained to discriminate modafinil from saline (Quisenberry and Baker, 2015). Although there are some studies that suggest that modafinil binds to NET in addition to DAT, the evidence is limited. For example, one study showed that modafinil displaced the binding of the radiolabeled NET ligand, [¹¹C](S,S)-2-(alpha-(2-methoxyphenoxy)benzyl)morpholine ([¹¹C]MeNER) in living primate brain (Madras et al., 2006). One interpretation of these data, however, is that in vivo binding of [¹¹C]MeNER is sensitive to endogenous NET substrates (Seneca et al., 2006), and there is a wealth of literature demonstrating the uptake of DA by NET (for review, see Carboni and Silvagni, 2004). Thus, it is conceivable that displacement of [¹¹C]MeNER is not necessarily evidence of direct binding of modafinil to NET and could be a consequence of elevated extracellular DA concentrations. In addition, other studies have replicated the finding that modafinil binds to DAT, but not to NET, in HEK293 cells and rat brain tissue (Mignot et al., 1994; Zolkowska et al., 2009; Loland et al., 2012; Nishino et al., 1998; Cao et al., 2011). Modafinil also did not have significant functional

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activity at blocking the reuptake of [³H]NE in rat brain tissue (IC₅₀>100,000 nM; Zolkowska et al., 2009). In contrast to modafinil, solriamfetol binds to and has activity at NET as demonstrated in the current binding, reuptake, and microdialysis studies. Further, solriamfetol dose-dependently inhibited hyperactivity in DAT knockout mice, suggesting that the activity of solriamfetol is not solely dependent on interactions at DAT (Jazz Pharmaceuticals, data on file). Thus, to the extent that NE is an important neurotransmitter involved in sleep-wake regulation (Berridge and Waterhouse, 2003; Berridge, 2008; Samuels and Szabadi, 2008; Szabadi, 2013), the dual activity of solriamfetol at DAT and NET might explain differences in the therapeutic effects of solriamfetol as compared with modafinil or armodafinil (Harsh et al., 2006; Schwartz et al., 2003; Ruoff et al., 2017).

The preclinical pharmacology studies presented in the current paper support the conclusion that the mechanism of action of solriamfetol involves low potency DA and NE reuptake inhibition via DAT and NET, respectively, but with distinct differences from stimulants (e.g. amphetamine) and reuptake inhibitors (e.g., cocaine and modafinil). Solriamfetol has wake-promoting effects in rodents (Hasan et al., 2009) and data from Phase 2/3 studies suggest solriamfetol has therapeutic potential for the treatment of ES and impaired wakefulness in patients with narcolepsy or OSA (Ruoff et al., 2016; Strohl et al., 2017; Strollo et al., 2017; Thorpy et al., 2017). Taken together, these data suggest that solriamfetol might offer an important advance in the treatment of ES in narcolepsy and OSA as well as other clinical conditions in which ES and impaired wakefulness would benefit from pharmacological intervention.

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Baladi M, Black J, Carter LP (2016) JZP-110, a dopamine norepinephrine reuptake inhibitor (DNRI), with robust wake-promoting effects and low abuse potential. Presented at: 141st Meeting of the American Neurological Association; October 16-18, 2016; Baltimore, MD. Poster M142.

Baladi M, Carter LP, Black J, Bergman J (2016) Evaluation of the effects of JZP-110 in nonclinical models of abuse liability. Presented at: 78th Annual Meeting of the College on Problems of Drug Dependence; June 11-16, 2016; Palm Springs, CA. Abstract 35. Oral presentation.

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Legends for Figures

Figure 1. Chemical structure of solriamfetol.

Figure 2. Effects of solriamfetol on release of [³H]monoamines in rat brain synaptosomes.

Various concentrations of solriamfetol were incubated with [³H]DA, [³H]5-HT, or [³H]NE in rat brain synaptosomes. Abscissa, concentration in μ M. Ordinate: mean expressed as a percentage of control reuptake determined from three separate experiments. Reference ligands were the following: for DAT, amphetamine; for NET, veratridine; and for SERT, 5-Methoxy-6-methyl-2-aminoindane. The concentrations of the reference ligands were the same as the concentrations for solriamfetol for each experiment. The corresponding clinically relevant concentration range in humans is shown within the shaded box.

Figure 3. Stimulus effects (panel A) and response rate (panel B) for solriamfetol in six rats discriminating 10 mg/kg cocaine from saline. Abscissae, dose of solriamfetol in mg/kg of body weight; open circles above control indicate the effects obtained with vehicle (saline) and solid gray squares indicate the effects obtained with 10 mg/kg cocaine. Ordinates: mean \pm SE percentage of responses on the cocaine lever (panel A) and mean \pm SE rate of responding (panel B).

Figure 4. Effects of acutely administered cocaine (panel A) and solriamfetol (panel B) on locomotor activity (n = 8 mice/group). Abscissa: dose of drug in mg/kg of body weight; open circles above control indicate the effects obtained with vehicle (saline). Ordinate: mean \pm SE

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ambulation counts during first 30 minutes. * $P < 0.05$. Unless shown, error bars are contained within the data symbol.

Figure 5. Effects of solriamfetol on DA (panel A) and NE (panel B) levels in rat striatum and prefrontal cortex, respectively, in microdialysis experiments (n = 12 rats/group). Abscissa: sampling period (minutes). The arrow indicates the time of s.c. administration of solriamfetol. Ordinate: mean \pm SE percentage change compared with each animal's average baseline value.

Table 1 Effects of solriamfetol in monoamine transporter binding assays

Binding Affinity (Ki or IC ₅₀)	Cell/Tissue Type					
	HEK293 cells ^a		Rat Brain Synaptosomes ^b (Striatum)		Rat Brain Synaptosomes ^c (Striatum)	
	Cocaine (nM)	Solriamfetol (nM)	Cocaine (nM)	Solriamfetol (nM)	Cocaine (nM)	Solriamfetol (nM)
DAT	236±58	14,200+3500	160 ^d	2,600 ^d	30 ^d	4100 ^d
NET	505±67	3700+1000	ND	ND	ND	ND
SERT	361±55	81,500+2900	ND	ND	ND	ND

^aRadioligand used was [125I]RTI-55; ^bRadioligand used was [3H]WIN 35438; ^cRadioligand used was [3H]Cocaine, ^dBinding affinity calculated as IC₅₀ rather than Ki. HEK, human embryonic kidney; ND, no data; DAT, dopamine transporter; NET, norepinephrine transporter; SERT, serotonin transporter. For experiments using cells, numbers represent the means ±SE from at least three independent experiments, each conducted with duplicate determinations.

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Table 2 Effects of solriamfetol in monoamine transporter reuptake inhibition and release assays

Reuptake Inhibition (IC ₅₀)	Cell/Tissue Type						
	HEK293 Cells		Rat Brain Synaptosomes ^a				
	Cocaine (nM)	Solriamfetol (nM)	GBR-12909 (nM)	Bupropion (nM)	Desipramine (nM)	Solriamfetol (nM)	
DAT [³ H]DA	385±54	2900±920	8	1700	ND	2±000	
NET [³ H]NE	194±29	4400±1100	ND	ND	4.2	6±000	
SERT [³ H]5-HT	355±39	>100,000	ND	ND	ND	ND	
Release (EC ₅₀) ^b	HEK293 Cells						
	Meth (nM)	PCA (nM)	Solriamfetol (nM)				
	DAT [³ H]DA	721±96	ND				NA
	NET [³ H]NE	103±35	ND				NA
SERT [³ H]5-HT	22,800±7000	430±120	NA				

^aRat brain striatum for DAT and hypothalamus for NET. ^bRelease (EC₅₀) testing performed only in HEK293 cells. DAT, dopamine transporter;

GBR-12909, (1-(2-(bis(4-fluorophenyl)-methoxy)-ethyl)-4-(3-phenyl-propyl)piperazine); HEK, human embryonic kidney; Meth,

methamphetamine; NA, a sigmoidal curve could not be fit to the data; ND, no data; NET, norepinephrine transporter; PCA, p-chloroamphetamine;

SERT, serotonin transporter. For experiments using cells, numbers represent the means \pm SE from at least three independent experiments, each conducted with triplicate determinations.

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Table 3. Effects of solriamfetol in receptor binding assays

Receptor Site	Ki (nM)
DA D ₁	>10,000
DA D ₂	>10,000
DA D ₃	>10,000
DA D ₄	>10,000
DA D ₅	>10,000
5-HT _{1A}	3558
5-HT _{2A}	>10,000
5-HT _{3A}	>10,000
5-HT _{2C}	>10,000
5-HT ₇	>10,000
α_{1A}	>10,000
α_{2A}	10,470
α_{2B}	2684
α_{2C}	>100,000
Histamine H ₁	>10,000
Histamine H ₂	>10,000
Histamine H ₃	>10,000
Neurokinin NK ₁	>10,000
nAChR	>10,000
CCK ₁	>10,000

CCK = cholecystokinin; nAChR = nicotinic acetylcholine receptors

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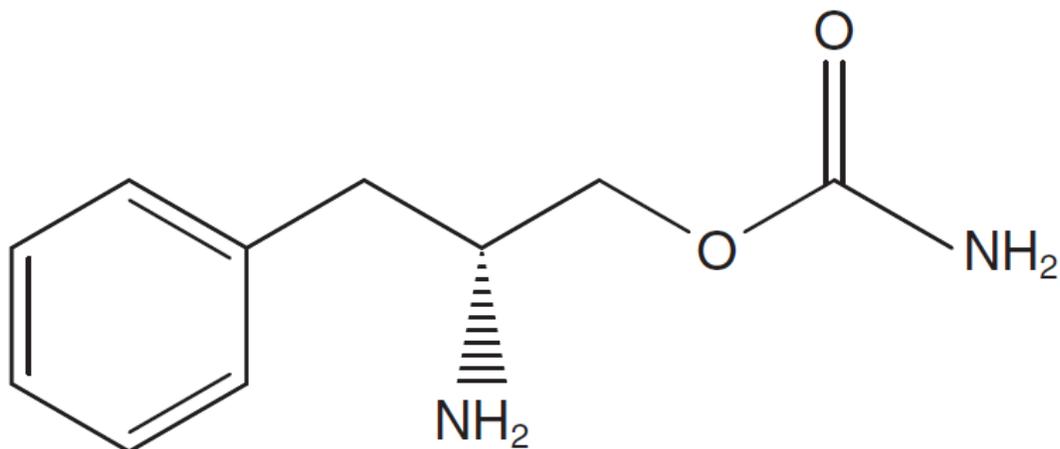


Figure 1

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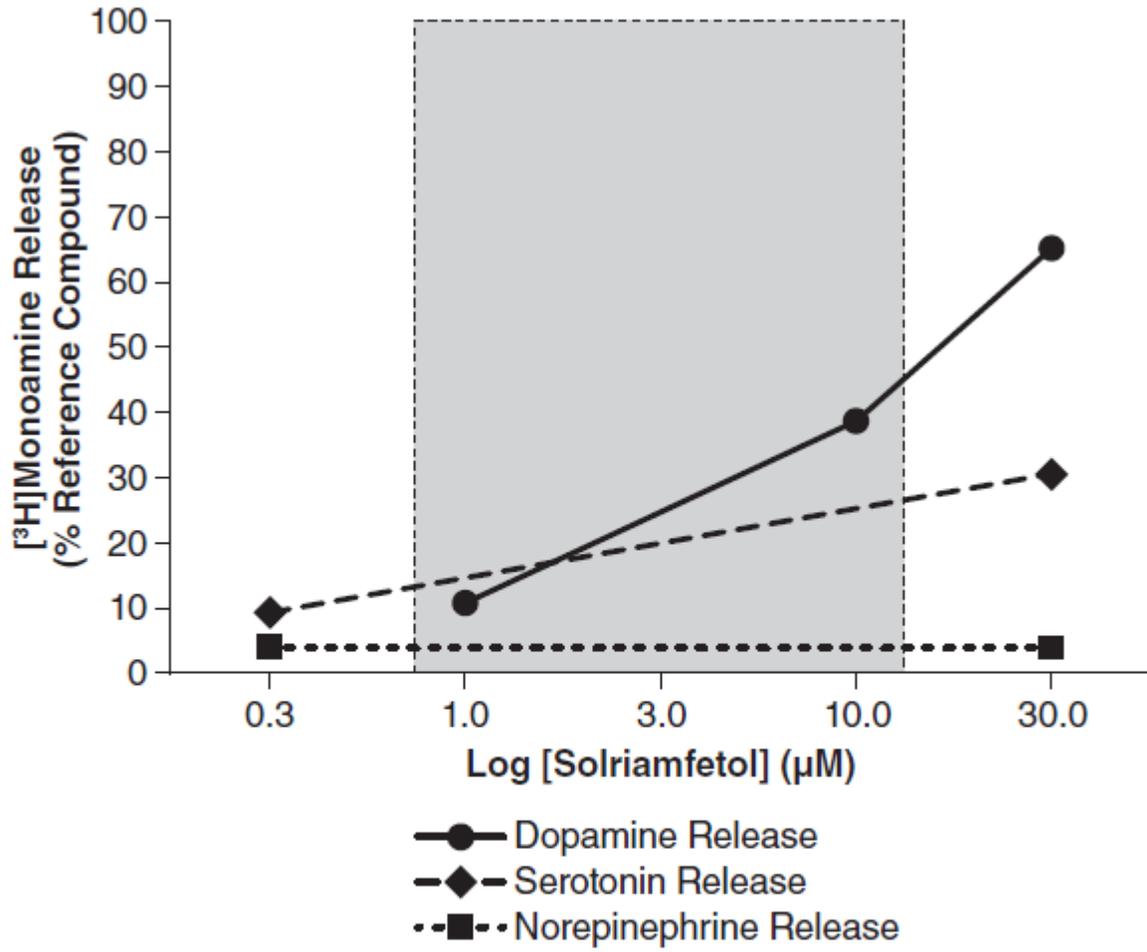


Figure 2

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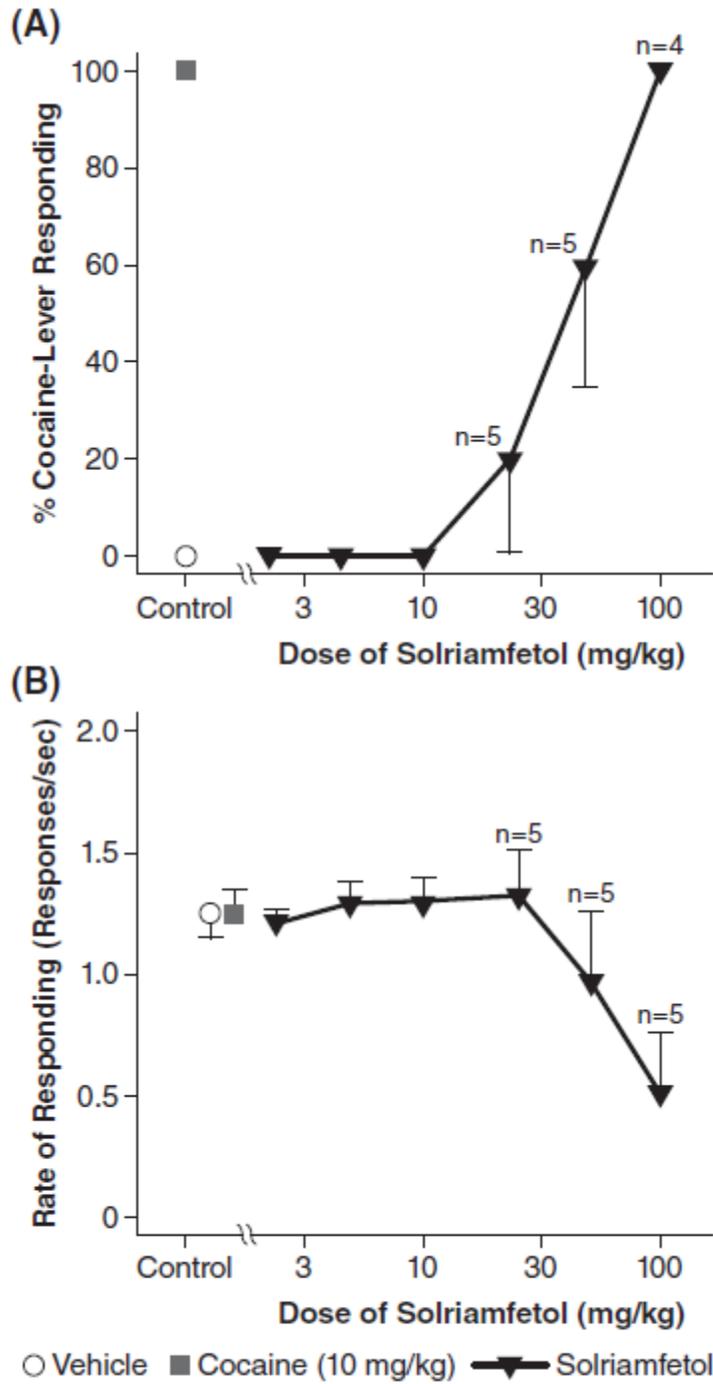


Figure 3

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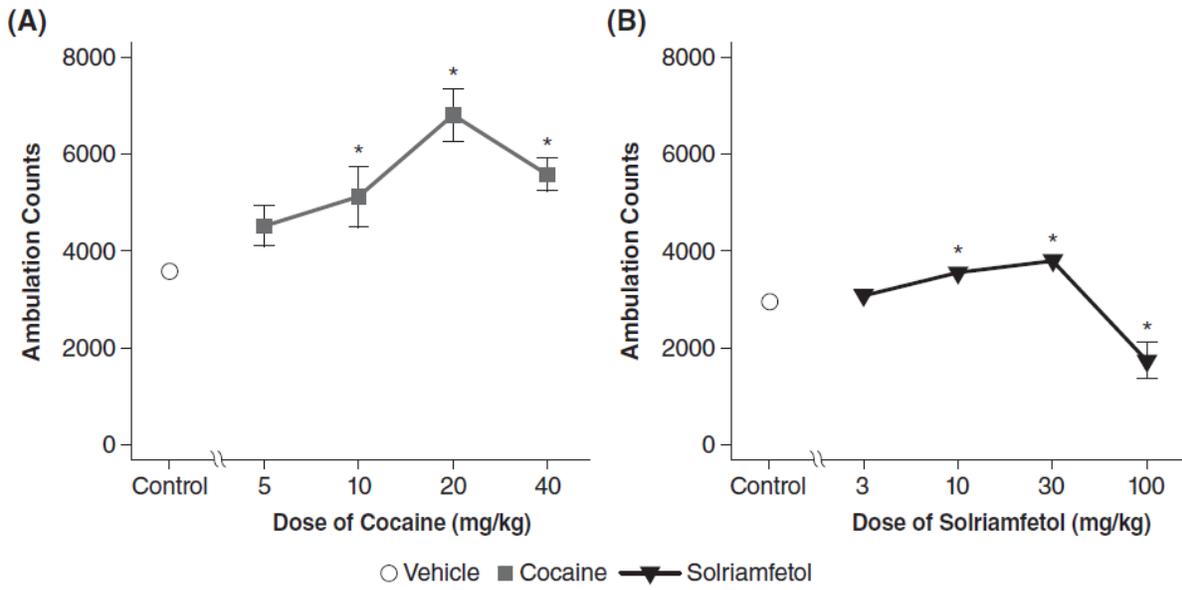


Figure 4

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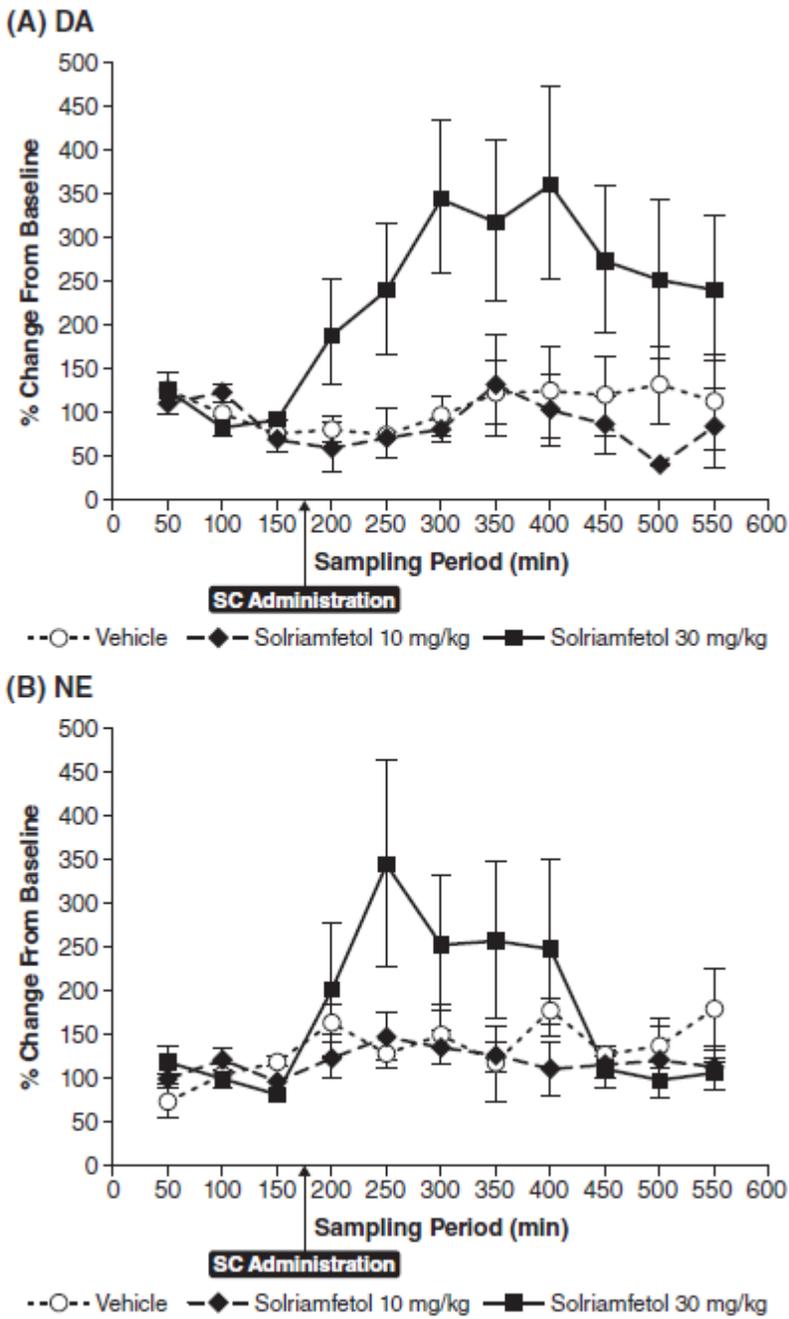


Figure 5