Characterization of Pharmacological and Wake-Promoting Properties of the Dopaminergic Stimulant Sydnocarb in Rats

John A. Gruner,1 Joanne R. Mathiasen,2 Dorothy G. Flood,3 and Maciej Gasior

CNS Biology, Worldwide Discovery Research, Cephalon, Inc., West Chester, Pennsylvania

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ABSTRACT

Sydnocarb is a psychomotor stimulant structurally similar to d-amphetamine (d-AMPH) and is used in Russia for the treatment of a variety of neuropsychiatric comorbidities. The nature of sydnocarb-induced facilitation of dopamine (DA) neurotransmission [DA release versus DA transporter (DAT) inhibition] is not clear. The present study characterized the pharmacological actions and behavioral effects of intraperitoneal sydnocarb in male Sprague-Dawley rats. Where relevant, comparisons were made with intraperitoneal d-AMPH. Unlike d-AMPH, which causes release of DA from rat synaptosomes (EC50 = 0.10 μM; 95% confidence limits, 0.06–0.18), sydnocarb (up to 100 μM) did not. Sydnocarb potently (Kᵢ = 8.3 ± 0.7 nM) blocked recombinant human DAT expressed in Chinese hamster ovary-K1 cells and less potently blocked the norepinephrine transporter (Kᵢ = 10.1 ± 1.5 μM). Sydnocarb at 10 μM did not bind to 64 other targets. In rats, 10 and 30 mg/kg sydnocarb showed a 2-fold longer half-life in plasma and brain and a 5-fold lower brain-to-plasma ratio compared with 0.3 and 1 mg/kg d-AMPH. In the Irwin assay, sydnocarb was well tolerated up to 30 mg/kg; d-AMPH-like stereotypic behaviors were evident at 100 mg/kg. Behavioral effects of 30 mg/kg sydnocarb and 0.3 mg/kg d-AMPH were comparable. In a sleep/wake assay, 10 mg/kg sydnocarb and 1 mg/kg d-AMPH increased wakefulness comparably; however, sydnocarb (up to 30 mg/kg) did not induce d-AMPH-like rebound hypersomnolence (RHS). Like d-AMPH, sydnocarb enhanced theta power, an electrophysiological measure of cognitive function. In conclusion, sydnocarb is a selective and potent DAT inhibitor that produces robust increases in the wake state without RHS, and with potential cognitive-enhancing properties.

Introduction

Sydnocarb [3-((β-phenylisopropyl)-N-phenylcarbamoylsydnonimine], also known as mesocarb, is a psychomotor stimulant structurally related and metabolized to d-AMPH (Fig. 1). Sydnocarb is banned by the World Anti-Doping Agency and scheduled in many countries (Cody, 2002; Docherty, 2008). In its country of origin, Russia, sydnocarb has been used to treat a variety of neuropsychiatric comorbidities such as asthenia, apathy, and adynamia (Anokhina et al., 1974; Vinar et al., 1991; Cody, 2002). Although mostly anecdotal, evidence suggests that sydnocarb increases endurance during heavy physical activity and resistance to environmental stressors such as hypothermia, low gravity, and oxygen deprivation; it may also have beneficial effects in treating alcohol abuse, attention deficit hyperactivity disorder, and cognitive impairment (Rudenko and Altshuler, 1979; Vinar et al., 1991; Cody, 2002).

Although sydnocarb-induced facilitation of dopamine (DA)-mediated transmission has been well established in microdialysis studies, the exact nature of this action [i.e., DA release versus DA transporter (DAT) inhibition] is not clear (Gainetdinov et al., 1997; Afnas’ev et al., 2001; Anderzhanova et al., 2001). There is evidence that sydnocarb attenuates noradrenaline reuptake based on experiments in rat synaptosomes (Erdő et al., 1981). Sydnocarb is also metabolized to d-AMPH in humans and animals (Fig. 1), but the role of d-AMPH in the net in vivo effects of sydnocarb is not known. In vivo pharmacological profiles of sydnocarb and d-AMPH

ABBREVIATIONS: d-AMPH, d-amphetamine; DA, dopamine; DAT, dopamine transporter; RHS, rebound hypersomnolence; EEG, electroencephalography; EMG, electromyography; ³H, tritium; MQL, minimum quantifiable limit; AUC, area under the curve; ZT, Zeitgeber time; REMS, rapid eye movement sleep; SWS, slow-wave sleep; CWT, cumulative wake time; CWS, cumulative wake surplus; ANOVA, analysis of variance; CHO, Chinese hamster ovary; NET, norepinephrine transporter; B/P, brain-to-plasma ratio; GBR-12909, 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenyl)propyl)piperazine; SERT, serotonin transporter; GBR-12935, 1-[2-(diphenylmethoxy)ethyl]-4-(3-phenyl)propyl)piperazine.
largely overlap, suggesting that either sydnocarb and D-AMPH are functionally indistinguishable or the metabolite D-AMPH contributes significantly to the effects produced by sydnocarb (Gainetdinov et al., 1997; Witkin et al., 1999; Anderzhanova et al., 2000; Flood et al., 2010). However, there are some important differences between the two drugs. Unlike D-AMPH, neither significant toxic episodes nor abuse potential have been reported with sydnocarb in humans (Mas'kovski et al., 1971; Rudenko and Altshuler, 1979). Compared with D-AMPH, the stimulating effects of sydnocarb develop more gradually, last longer, and are not accompanied by pronounced euphoria, motor excitation, or peripheral sympathomimetic effects such as tachycardia and hypertension (Rudenko and Altshuler, 1979). In animals, sydnocarb produces a slower and more gradual increase in extracellular DA in the rat striatum and nucleus accumbens compared with D-AMPH (Gainetdinov et al., 1997; Witkin et al., 1999; Anderzhanova et al., 2000). Relative to D-AMPH, equimolar doses of sydnocarb produce less hyperlocomotion and stereotypy as well as smaller changes in the markers of neurotoxicity such as DA depletion, generation of reactive oxygen species, or increases in specific indices of lipid peroxidation (Gainetdinov et al., 1997; Witkin et al., 1999; Anderzhanova et al., 2000; Afanas'ev et al., 2001; Bashkatova et al., 2002). Taken together, the available evidence suggests that sydnocarb may have a lower side-effect liability while retaining the core pharmacological spectrum of D-AMPH.

The present study was undertaken to investigate several key properties of sydnocarb to explain its pharmacological profile in vivo. First, the potential pharmacological mechanisms of action of sydnocarb were investigated by testing the compound’s ability to bind in vitro to a panel of 66 different ion channels, receptors, and neurotransmitter transporters. Second, the pharmacokinetic properties and behavioral side effects of sydnocarb were evaluated and compared with those of D-AMPH. Third, sydnocarb was tested for its locomotor-stimulant effects, wake-promoting effects, and propensity to produce rebound hypersomnolence (RHS) using electroencephalography/electromyography (EEG/EMG) sleep/wake methodology. RHS refers to “an intense interval of compensatory sleep after drug-induced waking” (Edgar and Seidel, 1997) and is a side effect of many wake-promoting agents—in particular, methamphetamine and D-AMPH (Tourret et al., 1995; Lin et al., 2000; Gruner et al., 2009). Fourth, EEG/EMG data were further analyzed for the effects of sydnocarb on EEG power in the theta frequency band (6–9 Hz). Increases in theta power have been considered to be an electrophysiological marker of increased functioning in cognitive domains such as learning, memory, or attention (see, for example, Başar et al., 2001; Caplan et al., 2003; Santos et al., 2008). Finally, the role of the metabolite D-AMPH in the wake-promoting activity of sydnocarb was assessed.

Materials and Methods

Animals. Male Sprague-Dawley rats (Charles River Laboratories, Inc., Wilmington, MA) were used for these studies. All animals were given food and water ad libitum and were maintained on a 12-h light/dark schedule, with lights on at 8:00 AM (sleep/wake studies) or 7:00 AM (all other studies). The experimental procedures were approved by the Cephalon, Inc., Institutional Animal Care and Use Committee and were in accordance with the guidelines in the Guide for the Care and Use of Laboratory Animals from the U.S. Department of Health and Human Services.

Drugs. The test compounds were sydnocarb (mol. wt. 322.37 g; synthesized by Value-Tek, Inc., Princeton, NJ) and D-amphetamine sulfate (mol. wt. 304.35 g; Sigma-Aldrich, St. Louis, MO). Compounds were formulated for administration using 0.5% methylcellulose (Methocel A15 Premium; Dow Chemical Company, Midland, MI)/0.2% Tween 80 (Thermo Fisher Scientific, Waltham, MA) in sterile water. Doses of D-AMPH referred to its salt form. For in vivo experiments, the compounds were administered in a volume of 5 ml/kg i.p.

Selectivity Assessment. Sydnocarb was tested for its binding properties to 66 pharmacologically relevant ion channels, receptors, and transporters according to standard validated protocols under conditions defined by the contractor (MDS Pharma Services, Taiwan Ltd., Taipei, Taiwan). Reference standards were run as an integral part of each assay to ensure the validity of the results obtained. Percent inhibition produced by 10 μM sydnocarb was first tested against all 66 targets. Then, concentration-effect functions for sydnocarb were evaluated for those targets that produced at least 50% inhibition in the initial screen. Inhibition constant (K_i) values were calculated using the observed concentration of sydnocarb that produced 50% inhibition (IC_{50} value), the concentration of the radiolabeled product's ability to bind in vitro to a panel of 66 different ion channels, receptors, and neurotransmitter transporters. The two hydroxylated metabolites were reported to be biologically inactive (Kholodov, 1981).
and used in the assay, and historical values for the \( K_t \) of the ligand obtained experimentally at MDS Pharma Services, Taiwan Ltd. (Cheng and Prusoff, 1973). \( K_t \) values were determined in triplicate and expressed as mean ± S.E.M.

In Vitro DA Release Experiments. \(^3\text{H}\)DA release was tested in \(^3\text{H}\)DA-prelabeled rat striatal synaptosomes in the presence of sydnocarb alone and in combination with d-AMPH. These procedures are described in detail elsewhere (Gruner et al., 2009). In brief, sydnocarb at various concentrations was first tested for its ability to induce \(^3\text{H}\)DA release. Then, the effects of 10 \( \mu \)M sydnocarb on d-AMPH-induced \(^3\text{H}\)DA release were evaluated by adding sydnocarb to the test synaptosomes in the presence of varying concentrations of d-AMPH. The effects of d-AMPH in the presence of 10 \( \mu \)M sydnocarb on \(^3\text{H}\)DA release were compared with the effects of d-AMPH alone. GraphPad Prism v4.00 (GraphPad Software Inc., San Diego, CA) was used to determine EC\(_{50}\) values using a sigmoidal dose-response curve fit (\( Y = \text{bottom} + (\text{top} - \text{bottom})/(1 + 10^{\text{LogEC}_{50} - X}) \)).

Irwin Behavioral Assay. The procedure was performed as previously described (Roux et al., 2004). In brief, after 30 min of acclimation to single housing in a clean observation cage, rats were administered compound with the observer blinded to the nature of the dosing solution except for an open vehicle control. Two control groups (one open, one blinded) were used to assess random variable compound effects. Sydnocarb (30 and 100 mg/kg i.p.) and d-AMPH groups (one open, one blinded) were used to assess random variable dosing solution effects. D-AMPH-induced \(^3\text{H}\)DA release were evaluated by adding sydnocarb to the test synaptosomes in the presence of varying concentrations of d-AMPH. The effects of d-AMPH in the presence of 10 \( \mu \)M sydnocarb on \(^3\text{H}\)DA release were compared with the effects of d-AMPH alone. GraphPad Prism v4.00 (GraphPad Software Inc., San Diego, CA) was used to determine EC\(_{50}\) values using a sigmoidal dose-response curve fit (\( Y = \text{bottom} + (\text{top} - \text{bottom})/(1 + 10^{\text{LogEC}_{50} - X}) \)).

Pharmacokinetic Profiling. Blood samples from the tail vein and brains were collected at various times ranging from 0.25 to 6 h after intraperitoneal dosing in rats (\( n = 3–6 \) rats/group; mean weight at dosing, 350 ± 10 g). Blood samples were centrifuged, and the plasma was collected and stored at −20°C. After each sample was collected, the animal was decapitated, and the brain was removed and homogenized with 2 volumes of 0.1 M phosphate-buffered saline, pH 7.2. The plasma samples and brain homogenate samples were prepared for bioanalysis by adding 10 volumes of acetonitrile containing an internal standard (alprenolol). After the samples were vortexed and centrifuged, the supernatant was analyzed by liquid chromatography/mass spectrometry. The amount of compound in the sample was quantified using a plasma (or brain homogenate) standard curve made via serial dilution in a concentration range from 5 to 5000 ng/ml. Plasma and brain concentrations were expressed as nanograms per milliliter and nanograms per gram, respectively, and their minimum quantifiable limits (MQL) were 5 ng/ml and 15 ng/g, respectively. WinNonlin Professional version 4.1 (Pharsight, Mountain View, CA) was used to calculate pharmacokinetic parameters such as maximal concentration (\( C_{\text{max}} \); nanograms per milliliter), time to achieve maximal concentration (\( t_{\text{max}} \) hours), exposure expressed as area under the curve (\( \text{AUC}_{0-\infty} \); nanograms per milliliter per hour), and half-life (\( t_{\frac{1}{2}} \) hours).

Sleep/Wake Activity. Sleep/wake activity was evaluated in rats using the standard methodology described in detail elsewhere (Gruner et al., 2009). Rats (\( n = 44 \) total; mean weight at dosing, 503 ± 6 g) were used for these studies.

EEG and EMG signals were recorded from chronically implanted screws in the skull and from intramuscular wires in the nuchal (dorsal neck) muscles, respectively. Animals were anesthetized with 45 mg/kg i.p. pentobarbital sodium (Nembutal; Abbott Laboratories, Abbott Park, IL) during surgery, and an anesthetic (2% lidocaine; Phoenix Pharmaceuticals, Phoenix, AZ) was used to minimize further discomfort. A sterilized temperature/activity transmitter (TA10TA-F40; Data Sciences International, St. Paul, MN) was implanted into the peritoneal cavity. Wound edges were treated with antibiotic cream (neomycin; Phoenix Pharmaceuticals), and a prophylactic antibiotic injection (0.2 ml × 50 mg/ml amikacin sulfate; Fort Dodge Laboratories, Overland Park, KS) was administered subcutaneously. Animals were allowed to recover from surgery for 2 weeks before recording, during which time they were housed in pairs in standard rat cages.

The day before a recording session, each rat was placed singly in a recording chamber and left undisturbed there until after the end of recording, except for dosing 5 h after lights on (Zeitgeber time (ZT) 5). EEG, EMG, and telemetry recordings were automatically initiated 2 h before dosing (ZT-3) and continued for 24 h, after which the animals were removed from the recording chambers and returned to their home cages. Dosing was by intraperitoneal route. Of the 66 tests, 18 involved repeat testing (15 animals tested twice, and 3 animals tested three times) with at least a 1-week washout between tests and with no animals receiving the same treatment twice.

The EEG and EMG data were classified into one of three sleep/wake states: wake, rapid eye movement sleep (REMS), and slow-wave sleep (SWS, also called non-REMS) according to semiautomated analysis of EEG frequency and amplitude characteristics and EMG activity. Sleep/wake stage scoring and analysis were performed by an investigator blinded to treatment. Waking activity was defined as consisting of relatively low-amplitude EEG activity with low power in the low-frequency bands from 0.3 to 6 Hz, accompanied by a moderate to high level of EMG activity. “Theta waking,” in which EEG power is relatively focused in the 6- to 9-Hz (theta) range and significant EMG activity is always present, was scored as wake. SWS was defined as generally high-amplitude EEG activity with greater power in the low-frequency bands from 0.3 to 6 Hz, accompanied by minimal EMG activity. REMS was characterized by a moderate and constant amplitude EEG concentrated in the theta range, similar to theta waking but with no EMG activity, and was preceded by SWS.

The principal outcome measures were percent time awake in consecutive 30-min periods and cumulative wake time (CWT) in minutes from the time of dosing. Corresponding calculations for SWS and REMS were also made. Cumulative awake surplus (CWS) produced by treatment was also calculated by subtracting the mean CWT value at each time point in the vehicle-treated group from the corresponding CWT value in the compound-treated group. Thus CWS represented the excess wake activity produced by the drug as a function of time compared with vehicle-treated animals.

Treatment efficacy was specifically compared using (1) percent time in wake, SWS (e.g., sleep efficiency), or REMS at various time points for compound-treated versus vehicle-treated groups; (2) cumulative time 4 h after dosing for wake, SWS, and REMS (4-h AUC); and (3) maximal CWS (within 10 h of injection). In addition, latencies to the onset of SWS and REMS were defined as the time from dosing to the detection of 2 consecutive min (20 epochs) of SWS and 1 consecutive min (10 epochs) of REMS, respectively. Finally, RHS was assessed after increased wake by comparing percent wake times between compound- and vehicle-treated groups. RHS was defined as occurring when the mean percent wake time in the compound-treated group was significantly less than that of the vehicle-treated group for a given time point.

EEG power in the theta band (6–9 Hz) was analyzed by Fourier analysis in 0.5-Hz frequency bins. Theta band center frequency (Hertz) and integrated power (millivolts squared per Hertz) were determined by separately averaging the wake and REMS epochs for intervals up to 4 h after dosing (Hajos et al., 2008). After determining the wake and REMS theta center frequency for each animal, theta power was integrated over the center frequency ± 2 Hz (five bins).

Motor Activity, Motor Intensity, and Body Temperature. Motor activity and body temperature were recorded using the Artquest (Data Sciences International) integrated hardware/software system. Each recording container was positioned above a receiver plate that detected the signal from the implanted transmitter. Average values were saved by the computer every 2 min. Motor activity included any movement causing the transmitter to be displaced relative to the receiver, such as locomotor activity and rearing.
Because motor activity can increase during increased wake, motor intensity was calculated to assess increases in motor activity independent from increased wake (Edgar and Seidel, 1997; Gruner et al., 2009). Motor intensity was calculated by dividing the average motor activity for the first 2 h after dosing by the corresponding mean time awake in minutes. An increase in motor intensity is indicative of the locomotor overstimulation resulting from the disproportionate increase in motor activity during increased wake. Both d-AMPH and methamphetamine have been reported to produce increases in motor intensity (Edgar and Seidel, 1997; Gruner et al., 2009). Body temperature was analyzed by calculating the mean body temperature for the 2 h before dosing and subtracting this value from all subsequent values, yielding the change from baseline.

**Statistics.** Treatment groups were compared using unpaired t tests or analysis of variance (ANOVA). A repeated measures ANOVA was used to compare wake curves across multiple time points. Post hoc tests were performed using unpaired t tests for the vehicle-treated group, a Dunnett’s multiple comparison test or the Holm-Sidak test for comparison to a single control group, or Bonferroni/Dunn tests for multiple comparisons across all treatment groups as appropriate (SigmaStat v3.5; SPSS Inc., Chicago, IL). Where appropriate, ED50 values (±S.E.M.) were calculated by using least-squares nonlinear regression (GraphPad Prism version 5.0 for Windows; GraphPad Software). Significance was set at p < 0.05 for all tests.

**Results**

**Pharmacological Action.** In the in vitro MDS Pharma ([125I]3β-(4’-iodophenyl)tropan-2β-carboxylic acid methyl ester binding assay, 10 µM sydnocarb produced 100% inhibition of DAT binding and 51% inhibition of NET binding. Sydnocarb at 10 µM produced less than 25% inhibition of the remaining 64 targets screened. In terms of function, sydnocarb was a potent and concentration-dependent inhibitor of human DAT (Ki = 8.3 ± 0.7 nM) recombinantly expressed in CHO-K1 cells (Fig. 2). Sydnocarb was approximately 1000-fold less potent at inhibiting human NET (Ki = 10.1 ± 1.5 µM) recombinantly expressed in CHO-K1 cells (Fig. 2).

**Effects of Sydnocarb and d-AMPH on DA Release.** Sydnocarb did not induce DA release up to 100 µM (Fig. 3A). At 10 µM, however, sydnocarb attenuated the ability of d-AMPH to induce DA release. This effect was evidenced by a significant rightward shift of the concentration-effect curves for d-AMPH in combination with sydnocarb compared with that for d-AMPH alone (Fig. 3B). As a result, the EC50 value of d-AMPH increased from 0.10 µM (95% confidence limits, 0.06–0.18) to 12.7 µM (4.0–40.8) in the presence of 10 µM sydnocarb (a 127-fold increase).

**Irwin Behavioral Assay.** Sydnocarb was evaluated at 30 and 100 mg/kg i.p. in the Irwin behavioral assay to estimate its therapeutic index and to establish its maximal safe dose for in vivo testing in rats. Sydnocarb at 30 mg/kg produced mild behavioral changes that included a slight increase in motor activity starting at 0.25 h, which progressed to slight sniffing at 0.5 h. By 1 h after injection, rats exhibited sniffing, rearing, and slight arousal that progressed to an increased startle response. From 2 to 6 h after injection, slight sniffing, rearing, arousal, increased tone, and mydriasis were evident. At 100 mg/kg, sydnocarb had more pronounced effects on behavior. Starting at 0.25 h after injection, sniffing, grip strength, arousal, pupil size, and locomotor activity were slightly increased. At 1 to 2 h after injection, the behavioral effects of sydnocarb intensified and in addition included irritability, biting, chewing, increased startle response, increased muscle tone, and increased urination. At 2 h after injection, behavioral effects became further pronounced and included dorsiflexion of the tail (Straub tail), ptosis, head bobbing, and increased body temperature. These behavioral effects remained unchanged up to 6 h after injection. All rats recovered and appeared normal 24 h after injections. Given these observations, the 100-mg/kg i.p. dose was deemed inappropriate for subsequent in vivo testing.

Testing of d-AMPH at 0.3, 1, 3, and 10 mg/kg i.p. was conducted for comparison. Behavioral effects produced by 0.3 mg/kg i.p. d-AMPH were similar to those produced by 30

![Fig. 2.](image-url) Mean ± S.E.M. (n = 3 per data point) percent inhibition of recombinant human DAT and NET by sydnocarb in CHO-K1 cells. Curves are the results of a least-squares fit of a sigmoidal concentration-response function (r² = 0.96 and 0.91 for curves at DAT and NET, respectively). The points of intersection of the concentration-response curves with the dashed line at 50% correspond to the calculated concentrations of sydnocarb that produced 50% inhibition (IC50 value) of DAT and NET activity, which were then used to calculate Kᵢ values (Cheng and Prusoff, 1973). Error bars for some of the data points are smaller than the data points.

![Fig. 3.](image-url) Effects of sydnocarb and d-AMPH on DA release in a rat synaptosome assay. Each plot shows mean ± S.E.M. (n = 3–9 per data point) percent of [3H]DA retained in synaptosomes after a 30-min incubation with test compounds. A, no effect of various concentrations of sydnocarb alone on [3H]DA release. B, the effect of various concentrations of d-AMPH alone (■) or in combination with 10 µM sydnocarb (□) on [3H]DA release. Solid curves represent sigmoidal dose-response curve fits. Error bars for some of the data points are smaller than the data points.
mg/kg i.p. sydnocarb. At 1 mg/kg i.p. d-AMPH, the effects intensified and included increased irritability, urination, grip strength, and vocalization to touch; all these behaviors were more intense at 3 mg/kg. At 10 mg/kg, d-AMPH-induced stereotypic behaviors were further enhanced with the addition of head weaving, chewing, tremor, head twitches, and exophthalmos; at 6 h after injection, stereotypic behaviors continued in one of three rats. All rats recovered and appeared normal 24 h after injection. The 3- and 10-mg/kg i.p. doses were deemed inappropriate for subsequent in vivo testing.

**Pharmacokinetic Properties of Sydnocarb in Comparison with d-AMPH.** A single administration of sydnocarb at 10 and 30 mg/kg i.p. resulted in a time- and dose-dependent exposure of the compound in the plasma and brain (Fig. 4; Table 1). The \( t_{max} \) after administration of sydnocarb at 10 and 30 mg/kg i.p. was 0.4 and 1.3 h, respectively; plasma \( t_{1/2} \) was 2.3 and 2.4 h, respectively. Brain to plasma ratios (B/P) ranged from 1.2 to 1.5 at 1 h and 6 h after injection of sydnocarb at both 10 and 30 mg/kg i.p. The brain \( t_{1/2} \) of sydnocarb after intraperitoneal administration at 10 and 30 mg/kg was 2.0 and 5.4 h, respectively.

Compared with sydnocarb (Table 1), the plasma \( t_{1/2} \) of d-AMPH was approximately 2-fold shorter and the B/P ratios were approximately 5-fold higher. The estimated brain \( t_{1/2} \) of 1 mg/kg i.p. d-AMPH was 1.1 h and was approximately 2- to 5-fold shorter than the values for sydnocarb at 10 and 30 mg/kg i.p., respectively.

Because sydnocarb is metabolized to d-AMPH, plasma and brain samples from rats treated with sydnocarb at 10 and 30 mg/kg i.p. were also screened for the presence of d-AMPH at 1, 2, 4, and 6 h after injection. No d-AMPH was detected in the plasma between 1 and 6 h after sydnocarb administration at 10 and 30 mg/kg i.p. No d-AMPH was detected in the brain between 1 and 6 h after administration of 10 mg/kg sydnocarb; however, d-AMPH was detected in brain at a concentration of 67 ± 49 ng/g (n = 3) only at 2 h after 30-mg/kg sydnocarb treatment. For comparison, brain concentrations of 259 ± 23 ng/g (n = 4) were measured in the brain 1 h after administration of 0.3 mg/kg i.p. d-AMPH. d-AMPH at 1 mg/kg i.p. produced brain concentrations of 884 ± 32, 139 ± 15, and 34 ± 6 (n = 4) at 1, 3, and 6 h after injection, respectively.

**Effects of Sydnocarb on Sleep/Wake Activity.** Sydnocarb was tested at 1, 3, 10, and 30 mg/kg i.p., and d-AMPH was tested at 0.3 and 1 mg/kg i.p. in parallel for comparison. Sydnocarb and d-AMPH produced dose-related increases in wake (Figs. 5 and 6; Table 2) as revealed by significant increases in time spent in wake (\( F_{6,57} = 99.58, p < 0.001 \)), with corresponding increases in latencies to SWS (\( F_{6,57} = 69.89, p < 0.001 \)) and REMS (\( F_{6,57} = 58.17, p < 0.001 \)) and decreases in durations of SWS (\( F_{6,57} = 81.42, p < 0.001 \)) and REMS (\( F_{6,57} = 31.62, p < 0.001 \)). Wake was increased by sydnocarb at 3, 10, and 30 mg/kg i.p. compared with vehicle-treated animals (\( p < 0.05 \), Dunnett’s test; Figs. 5 and 6). Likewise, latencies to SWS and REMS increased significantly after sydnocarb administration at 10 and 30 mg/kg i.p. (\( p < 0.05 \), Dunnett’s test) compared with those of vehicle-treated animals (Table 2). Time spent in SWS and REMS in the first 4 h after dosing was significantly decreased by sydnocarb at 3 to 30 and 10 to 30 mg/kg i.p., respectively (\( p < 0.05 \), Dunnett’s test; Fig. 5); sydnocarb’s ED\(_{50}\) values for reducing SWS and REMS were 5.7 (±1.2) and 4.5 (±1.3) mg/kg, respectively.

d-AMPH increased wake at each dose studied, 0.3 and 1.0 mg/kg i.p. (\( p < 0.05 \), Dunnett’s test). Increases in wake produced by 1 mg/kg i.p. d-AMPH were comparable with those produced by 10 mg/kg i.p. sydnocarb. In particular, 1 mg/kg i.p. d-AMPH and 10 mg/kg i.p. sydnocarb increased wake by the same amount up to 3 h after dosing (\( p > 0.05 \), Bonferroni t test; Figs. 5 and 7). Likewise, latencies to the onset of SWS and REMS were not different at these doses (\( p > 0.05 \), Bonferroni t test; Table 2).

Sydnocarb did not produce RHS at any dose. After the period of enhanced wake, activity was comparable with the normal circadian activity in the vehicle-treated group. However, there was an episode of decreased wake in the 10-mg/kg i.p. sydnocarb group at 10.5 h after dosing (\( p < 0.05 \) versus vehicle, unpaired t test). In contrast, 1 mg/kg i.p. d-AMPH produced RHS between 4 and 6 h after dosing as evidenced by

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**Table 1**

**Pharmacokinetic profile of sydnocarb compared with d-AMPH**

<table>
<thead>
<tr>
<th>Sydnocarb</th>
<th>d-AMPH</th>
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<tbody>
<tr>
<td>10 mg/kg</td>
<td>30 mg/kg</td>
</tr>
<tr>
<td>( C_{max} ) (ng/ml)</td>
<td>1030 ± 120</td>
</tr>
<tr>
<td>( t_{max} ) (h)</td>
<td>0.38 ± 0.12</td>
</tr>
<tr>
<td>AUC(_{0-\infty}) (ng · h⁻¹ · ml⁻¹)</td>
<td>3100 ± 430</td>
</tr>
<tr>
<td>( t_{1/2} ) (h)</td>
<td>2.29 ± 0.17</td>
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<tr>
<td>B/P (1 h)</td>
<td>1.39 ± 0.04</td>
</tr>
<tr>
<td>B/P (6 h)</td>
<td>1.22 ± 0.09</td>
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AUC\(_{0-\infty}\), area under the curve until 6 h after dosing; B/P (1 h) and B/P (6 h), ratios of brain-to-plasma concentrations at 1 and 6 h after injection, respectively; N.A., not available because of concentrations of d-AMPH below the MQL of 5 ng/ml and 15 ng/g in the plasma and brain, respectively.
approximately 20% less wake during this period compared with the vehicle-treated group ($p < 0.05$, unpaired $t$ test; Fig. 7). A lower dose of d-AMPH, 0.3 mg/kg i.p., showed the same magnitude of RHS as d-AMPH at 1.0 mg/kg, but the effect did not reach significance because of the small group size ($n = 4$).

A comparison of the maximum CWS produced by sydnocarb (1–30 mg/kg i.p.) and d-AMPH (1 mg/kg i.p.) up to 22 h after dosing is presented in Fig. 8. In this graph, a positive slope indicates increasing time spent awake compared with vehicle, and a negative slope indicates sleep recovery. Relative to the vehicle-treated group, sydnocarb produced maximum CWS values of 126 min at 4.5 h after dosing at 10 mg/kg and 270 min at 8.5 h after dosing at 30 mg/kg. The maximum CWS for d-AMPH at 1 mg/kg was 110 min at 3.5 h after dosing, which was not different from the maximum CWS for 10 mg/kg i.p. sydnocarb ($p > 0.05$, unpaired $t$ test). However, the d-AMPH group showed a more rapid loss of wake surplus than the sydnocarb group, which was consistent with RHS produced by 1.0 mg/kg i.p. d-AMPH. The CWS values for 10 mg/kg i.p. sydnocarb were greater than for 1 mg/kg d-AMPH from 5 to 10.5 h after dosing ($p < 0.05$, unpaired $t$ test). Thus, 10 mg/kg i.p. sydnocarb maintained 47 min more wake than 1 mg/kg d-AMPH at 10.5 h after dosing, despite comparable wake-promoting activities up to 3.5 h after dosing. Sydnocarb at 30 mg/kg maintained CWS for over 22 h after dosing. At this dose, from the time of maximum CWS (8.5 h after dosing) to the end of recording (22 h after dosing), CWS declined from 270 to 167 min, giving a sleep recovery rate of 7.7 min/h.

The theta frequency band was analyzed in terms of a change in absolute theta power and theta center frequency during periods of wake. Absolute theta power was increased by sydnocarb at 3, 10, and 30 mg/kg (Table 3), with a maximal increase in magnitude of approximately 212% and duration of increase up to 6 h after dosing with 30 mg/kg sydnocarb. d-AMPH (1 mg/kg) increased the absolute theta power by approximately 146% for up to 3 h after dosing. When equal wake-promoting doses of sydnocarb (10 mg/kg) and d-AMPH (1 mg/kg) are compared, both drugs significantly increased theta, but the effect of sydnocarb lasted nearly twice as long as that produced by d-AMPH (Table 3).

Untreated rats showed a theta center frequency of 7 Hz during REMS (Fig. 9A) and 6.0 to 6.5 Hz during wake (Fig. 9B). Theta center frequency was increased by sydnocarb at 1, 3, and 30 mg/kg for 20 to 60 min after dosing (Fig. 9, D and F; Table 3), and up to 2 to 3 h after sydnocarb administration at 10 mg/kg (Fig. 9E; Table 3). d-AMPH (1 mg/kg) right-shifted the wake-theta center frequency from 20 to 60 min up to 3 h after dosing (Fig. 9C; Table 3). The doses producing comparable wake increases, 10 mg/kg sydnocarb and 1 mg/kg d-AMPH, also produced generally comparable shifts in the direction, duration, and magnitude of theta center frequency ($p < 0.05$).
Motor Activity, Motor Intensity, and Body Temperature. Both sydnocarb and d-AMPH increased motor activity ($F_{5.42} = 8.466, p < 0.001$; Fig. 10A). Significant increases were produced by sydnocarb at 10 and 30 mg/kg i.p. and d-AMPH at 1 mg/kg i.p. ($p < 0.05$, Dunnett's test). In contrast, sydnocarb had no effect on motor intensity ($F_{4.41} = 0.532, P > 0.05$), whereas 1 mg/kg i.p. d-AMPH significantly increased motor intensity ($p < 0.05$, Dunnett's test; Fig. 10B).

Sydnocarb and d-AMPH time-dependently affected the body temperature ($F_{5.42} = 5.037, p = 0.001$) up to the end of the light phase (7 h after dosing; Fig. 10C). In particular, sydnocarb increased temperature for up to 1 h after dosing at 1 mg/kg, from 1 to 5 h at 3 mg/kg, from 3 to 5 h at 10 mg/kg, and from 3 to 7 h at 30 mg/kg ($p < 0.05$, Dunnnett's test). d-AMPH increased temperature from 1 to 4 h at 1 mg/kg ($p < 0.05$, Dunnett's test). Thus, d-AMPH at 1 mg/kg had an effect similar to that produced by sydnocarb at 3 mg/kg.

**Discussion**

Sydnocarb is regarded as a d-AMPH-like dopaminergic agent, but its mechanism of action has not been rigorously evaluated. In the present study, sydnocarb acted primarily as a potent and selective DAT inhibitor lacking any d-AMPH-like ability to release DA in vitro. Sydnocarb exhibited...
D-AMPH-like psychostimulatory effects in the Irwin behavioral assay. However, sydnocarb exhibited robust wake-promoting properties, which were both quantitatively and qualitatively distinguishable from those of D-AMPH. In particular, sydnocarb was more efficacious than D-AMPH in promoting wake and did not produce D-AMPH-like RHS and increased motor intensity. Both RHS and increased motor intensity are side effects of D-AMPH-like psychostimulants (Edgar and Seidel, 1997). Like D-AMPH, sydnocarb increased the EEG theta power, an effect that has been suggested to reflect an enhancement of procognitive functions (McNaughton et al., 2007; Hajós et al., 2008). Moreover, sydnocarb favorably differed from D-AMPH on the key pharmacokinetic properties that contribute to side-effect liabilities of dopaminergic psychostimulants as discussed below. Finally, metabolism of sydnocarb to pharmacologically relevant concentrations of D-AMPH was confirmed. However, the role of D-AMPH in the wake-promoting effects of sydnocarb in at least rats was ruled out based on comparisons of the brain concentrations of

- Fig. 9. Fast-Fourier transform (FFT power, millivolts squared per Hertz) of EEG at select time intervals before and after dosing with sydnocarb or D-AMPH. A, average power before and after dosing with vehicle during REMS epochs. Note that the specific increase in theta band (6–9 Hz) power. B, similar to A, but for wake epochs. C–F, theta power during wake epochs for D-AMPH (C, 1 mg/kg) and sydnocarb (D, 3 mg/kg; E, 10 mg/kg; and F, 30 mg/kg). Theta power for 1 mg/kg sydnocarb (not shown) was not different from vehicle. Time interval legends for plots B–F are the same as in A. Both sydnocarb and D-AMPH increased theta amplitude and center frequency.

- Fig. 10. Effect of sydnocarb (1, 3, 10, and 30 mg/kg i.p.) and D-AMPH (1.0 mg/kg) on motor activity and body temperature (mean ± S.E.M.). A, motor activity measured cumulatively in arbitrary units over 4 h after dosing. B, motor intensity (motor activity divided by the time spent in wake over 2 h after dosing; see Edgar and Seidel, 1997). C, temperature change (in Celsius) from the baseline at 1 to 7 h after dosing. The baseline temperature was established by averaging temperatures recorded at 1 and 2 h before treatment in each rat separately. Values in parentheses in the legend represent time points at which a given treatment produced a significant increase in body temperature compared with the vehicle-treated group at the corresponding post-treatment time point. The number of animals per treatment group was 17 (vehicle), 6, 7, 9, and 7 (sydnocarb at 1, 3, 10, and 30 mg/kg, respectively), and 6 (D-AMPH at 1 mg/kg). *p < 0.05; Dunnett’s test versus vehicle following significant ANOVA. veh, vehicle.
Sydnocarb lacked the D-AMPH-like ability to promote DA release in vitro. In contrast, sydnocarb potently inhibited DAT at low nanomolar concentrations and blocked NET at micromolar concentrations. Moreover, sydnocarb attenuated the ability of D-AMPH to induce DA release by 127-fold. This action of sydnocarb was consistent with the effects of other DAT inhibitors in the same synaptosomal assay (Gruner et al., 2009). Except for actions at DAT and NET, sydnocarb showed no activity at 64 other targets.

The pharmacological action of sydnocarb appeared more consistent with DAT inhibitors such as cocaine, methylenephendate, 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine (GBR-12909), or nomifensine than it did with DA-releasing agents such as D-AMPH, methamphetamine, or phentermine (Rothman and Baumann, 2003). All prototypical DAT inhibitors showed differential actions at NET and serotonin (SERT), whereas sydnocarb was the most selective DAT inhibitor (Table 4). A qualitatively similar profile of relative actions at NET and SERT versus DAT was demonstrated only by GBR-12909 and 1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)-piperazine (GBR-12935), with sydnocarb showing still greater selectivity.

**Wake-Promoting Effects.** Like other agents that increase DA transmission (Nishino et al., 1998; Gruner et al., 2009), sydnocarb increased wake as evidenced by increases in onset latency and decreases in the duration of SWS and REMS. Sydnocarb was effective from 3 to 30 mg/kg; 10 mg/kg sydnocarb and 1 mg/kg D-AMPH produced comparable increases in wake up to 3 h after dosing.

Sydnocarb at 100 mg/kg and D-AMPH at 3 mg/kg produced marked behavioral side effects that were determined unacceptable for in vivo testing. Thus, the doses of sydnocarb and D-AMPH that produced equal wake promotion (10 and 1 mg/kg, respectively) were 10- and 3-fold lower, respectively, than the doses that produced behavioral side effects in the Irwin behavioral test. This somewhat better side-effect profile for sydnocarb compared with other dopaminergic agents has been reported elsewhere (Witkin et al., 1999; Anderzhanova et al., 2000).

**Rebound Hypersomnolence.** Sydnocarb also differed from D-AMPH with regard to the propensity to produce RHS. In particular, sydnocarb did not produce RHS at any dose tested up to 10 h after dosing. In contrast, D-AMPH and methamphetamine produced RHS (present study; Touret et al., 1995; Edgar and Seidel, 1997; Gruner et al., 2009).

RHS is a side effect of some wake-promoting agents—in particular, DA-releasing agents—that limits their clinical efficacy (Touret et al., 1995; Edgar and Seidel, 1997; Lin et al., 2000). However, the involvement of DAT inhibition in RHS is not consistent. As recently reported (Gruner et al., 2009), some DAT inhibitors (e.g., cocaine, bupropion, and methylphenidate) produce RHS after the onset of sleep recovery, whereas others (e.g., mazindol, nomifensine, GBR-12909, and GBR-12935) do not produce RHS at doses that were equally wake promoting. The reason for this discrepancy is not clear and may be a result of some specific pharmacologic and/or pharmacokinetic properties of different DAT inhibitors (Gruner et al., 2009).

One factor that might affect RHS is the compound’s effect on motor activity. Because a number of DA releasers and DAT inhibitors are psychomotor stimulants, it can be argued that excessive motor activation could either prevent animals from going to sleep or lead to exhaustion and subsequent rapid sleep recovery manifesting as RHS. This presumption is unlikely in the present study because sydnocarb at 10 and 30 mg/kg and D-AMPH at 1 mg/kg produced comparable motor activation despite different propensities to produce RHS. A similar lack of correlation between motor-activating effects and the propensity to produce RHS was true for a number of DAT inhibitors (Gruner et al., 2009). In addition, although sydnocarb and D-AMPH each increased motor ac-

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**TABLE 4**

Comparison of $K_i$ values at DAT, NET, and SERT

Propensity of sydnocarb, various DAT inhibitors, and D-AMPH to produce RHS (present study; Gruner et al., 2009) and their estimated $K_i$ values at DAT, NET, and SERT in mice or rats (Pan et al., 1994; Nishino et al., 1998; Rothman and Baumann, 2003; Han and Gu, 2006). Values in parentheses represent ratios of $K_i$ values at NET or SERT over $K_i$ values at DAT.

<table>
<thead>
<tr>
<th>Compound</th>
<th>RHS</th>
<th>DAT $K_i$ (nM)</th>
<th>NET $K_i$ (nM)</th>
<th>SERT $K_i$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sydnocarb</td>
<td>No</td>
<td>8.3</td>
<td>1560 (181)</td>
<td>&gt;10,000 (&gt;1205)</td>
</tr>
<tr>
<td>GBR-12909</td>
<td>No</td>
<td>4.3</td>
<td>79.2 (18.4)</td>
<td>73.2 (17.0)</td>
</tr>
<tr>
<td>GBR-12935</td>
<td>No</td>
<td>4.9</td>
<td>277 (56.5)</td>
<td>289 (59.0)</td>
</tr>
<tr>
<td>Mazindol</td>
<td>No</td>
<td>25.9</td>
<td>2.9 (0.1)</td>
<td>272 (10.5)</td>
</tr>
<tr>
<td>Nomifensine</td>
<td>No</td>
<td>93.1</td>
<td>32 (0.3)</td>
<td>1888 (20.3)</td>
</tr>
<tr>
<td>Cocaine</td>
<td>Yes</td>
<td>478</td>
<td>779 (1.6)</td>
<td>304 (0.6)</td>
</tr>
<tr>
<td>Methylphenidate</td>
<td>Yes</td>
<td>260</td>
<td>170 (0.7)</td>
<td>1143 (4.4)</td>
</tr>
<tr>
<td>Bupropion</td>
<td>Yes</td>
<td>1534</td>
<td>&gt;10,000 (&gt;6.5)</td>
<td>34,707 (22.6)</td>
</tr>
<tr>
<td>D-AMPH</td>
<td>Yes</td>
<td>550</td>
<td>120 (0.2)</td>
<td>2382 (4.3)</td>
</tr>
</tbody>
</table>
activity, sydnocarb increased motor activity in proportion to wake activity as measured by motor intensity. In contrast, d-AMPH (present study; Gruner et al., 2009) produced increases in locomotor activity that were disproportionately high relative to the increases in wake. Similar increases in motor intensity were reported for methamphetamine (Edgar and Seidel, 1997).

**Theta Activity.** Sydnocarb increased the magnitude and duration of theta power in a dose-dependent manner. Effects of sydnocarb at 10 mg/kg and d-AMPH were comparable. There was further increase in the magnitude and duration of theta activity produced by sydnocarb at 30 mg/kg that was well tolerated in the Irwin behavioral assay. These findings are consistent with the effect reported in the literature for d-AMPH (Young, 1988; Hasan et al., 2009) and the DAT-inhibiting agent methylphenidate (Hajós et al., 2008). Consistent with sydnocarb's molecular action at NET, selective NET inhibitors have also been reported to either induce or enhance theta activity (Hajós et al., 2003a). Similar theta-enhancing effects were reported for agents with established cognitive-enhancing properties such as nicotine (Lu and Henderson, 2010) or acetylcholine esterase inhibitors (Dimpfel, 2005), as well as for agents from a variety of pharmacological classes being developed as the next generation of cognitive-enhancing drugs, including histamine 3 receptor antagonists/inverse agonists (Hajós et al., 2008), \( \alpha_1 \)-nicotinic acetylcholine receptor agonists (Siok et al., 2006), 5-hydroxytryptamine 1A receptor agonists (Marrous et al., 1996), and 5-hydroxytryptamine 2C receptor antagonists (Hajós et al., 2003b). Thus the theta-enhancing effect of sydnocarb reported in the present study warrants further systematic evaluation of its effects on cognition (McNaughton et al., 2007).

The propensity of sydnocarb and several DAT inhibitors to produce RHS and their relative actions at other monoamine transporters is compared in Table 4. Compounds with higher affinities for DAT (i.e., \( K_i = 4.3-93.1 \text{ nM} \)) do not produce RHS, whereas compounds with relatively lower affinities (\( K_i = 260-1534 \text{ nM} \)) do produce RHS. Furthermore, sydnocarb differed from all prototypical DAT inhibitors in that it showed the highest selectivity at DAT versus NET or SERT (Table 4). However, this property by itself cannot explain the lack of RHS in the case of sydnocarb, because mazindol and nomifensine also did not induce RHS despite lacking high relative selectivity at DAT compared with NET and SERT (Gruner et al., 2009).

**Pharmacokinetic Properties of Sydnocarb.** The net in vivo effects produced by compounds depend on their specific pharmacodynamic actions as much as on their unique pharmacokinetic properties. In the case of agents affecting DA neurotransmission, those that produce faster and larger changes in synaptic DA concentration are generally associated with a higher propensity for producing side effects (Swanson and Volkow, 2002; Lile, 2006; Berman et al., 2009).

As demonstrated in the present study, the pharmacokinetic properties of sydnocarb may contribute to its favorable net pharmacological profile, including prolonged wake activity without d-AMPH-like RHS and increased motor intensity. Compared with d-AMPH, sydnocarb showed longer plasma and brain exposure, and either comparable or later \( t_{\text{max}} \). Such a pharmacokinetic profile is consistent with the effects of sydnocarb on DA levels reported in microdialysis studies, where increases in DA concentrations produced by sydnocarb were characterized by a slower onset, lower magnitude, and longer duration compared with d-AMPH or methamphetamine (Gainetdinov et al., 1997; Witkin et al., 1999; Andersonova et al., 2000; Afanas'ev et al., 2001).

**Sydnocarb and its Metabolite d-AMPH.** It has recently been reported that prolonged waking without RHS can be achieved by coadministering the DAT inhibitor nomifensine and d-AMPH (Gruner et al., 2009). Because sydnocarb is metabolized to d-AMPH, the role of d-AMPH in the net pharmacological profile of sydnocarb on wake measures should be considered. No d-AMPH was detected in the brain within 1 to 6 h after administration of 10 mg/kg sydnocarb; for example, d-AMPH at the equally wake-promoting dose, 1 mg/kg, produced brain concentrations of 884 ng/g. Furthermore, d-AMPH was only detected in the brain 2 h after 30-mg/kg sydnocarb treatment at a concentration that was approximately 4-fold lower than that produced by the lowest dose of d-AMPH (0.3 mg/kg i.p.), which enhanced wake in this assay (present study; Gruner et al., 2009). The present study demonstrated that sydnocarb was metabolized to d-AMPH in rats but the concentrations of d-AMPH were low compared with those produced by wake-promoting doses of d-AMPH. Therefore, d-AMPH seems unlikely to play a major role in the wake-promoting effects of sydnocarb, at least in rats. Nevertheless, the concentration of d-AMPH in the brain (0.5 \( \mu \text{M} \)) metabolized from 30 mg/kg i.p. sydnocarb was within the range of the EC\(_{50}\) value for d-AMPH (0.095 \( \mu \text{M} \)) for DA release from the rat synaptosomes (Gruner et al., 2009). Thus, a role of d-AMPH in other behavioral effects of sydnocarb in Sprague-Dawley rats, as observed in the Irwin behavioral assay, cannot be ruled out. Even so, in the presence of a potent DAT inhibitor (sydnocarb), the propensity of d-AMPH to produce large increases in extracellular DA would be mitigated, as demonstrated by the ability of sydnocarb and other DAT inhibitors to decrease the ability of d-AMPH to induce DA release from rat synaptosomes (present study; Gruner et al., 2009).

In conclusion, sydnocarb is a potent and selective DAT inhibitor that displays pharmacokinetic and pharmacological properties that favorably distinguish it from the prototypical dopaminergic stimulant, d-AMPH, in regard to wake promotion, RHS, cognitive enhancement, and therapeutic index in experimental animals. That sydnocarb, like several other selective DAT inhibitors, lacks the propensity to induce d-AMPH-like RHS further implicates DA release as a causative mechanism responsible for RHS.

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**Authorship Contributions**

**Participated in research design:** Gruner, Mathiasen, Flood, and Gasior.

**Conducted experiments:** Gruner and Mathiasen.

**Contributed new reagents or analytic tools:** Gruner.

**Performed data analysis:** Gruner, Mathiasen, and Gasior.

**Wrote or contributed to the writing of the manuscript:** Gruner, Mathiasen, Flood, and Gasior.
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Address correspondence to: Dr. Maciej Gasior, Clinical Research, CNS/Pain, Cephalon, Inc., 41 Moores Rd., Frazer, PA 19355. E-mail: mgasior@cephalon.com