Modafinil Occupies Dopamine and Norepinephrine Transporters in Vivo and Modulates the Transporters and Trace Amine Activity in Vitro


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ABSTRACT

2-[(Diphenylmethyl) sulfinyl]acetamide (modafinil), prescribed principally to treat narcolepsy, is undergoing assessment for other neuropsychiatric disorders and medical conditions. The neurochemical substrates of modafinil are unresolved. We postulated that modafinil enhances wakefulness by modulating dopamine (DAT), norepinephrine (NET), or serotonin (SERT) transporter activities. In vivo, we determined DAT and NET occupancy by modafinil by positron emission tomography imaging; in vitro, we determined modafinil activity at the DAT, NET, and rhesus monkey trace amine receptor 1 (TA1). In rhesus monkey, modafinil occupancy of striatal DAT was detected by $[^{11}C]2$-[(2-methoxyphenoxy)benzyl)morpholine. In vitro, modafinil effects in DAT-human embryonic kidney (HEK), NET-HEK, and SERT-HEK cells were investigated alone or combined with the TA1 receptor. Modafinil (i.v.) occupied striatal DAT sites (5 mg/kg: $35 \pm 12\%$, $n = 4$; 8 mg/kg: $54 \pm 33\%$, $n = 3$). In thalamus, modafinil occupied NET sites (5 mg/kg: $16 \pm 7.8\%$, $n = 6$; 8 mg/kg: $44 \pm 12\%$, $n = 2$). In vitro, modafinil inhibited $[^{3}H]$dopamine (IC$_{50} = 6.4 \mu M$), $[^{3}H]$norepinephrine (IC$_{50} = 35.6 \mu M$), and $[^{3}H]$serotonin (IC$_{50} > 500 \mu M$) transport via the human DAT, NET, and SERT. Modafinil did not activate the TA1 receptor in TA1-HEK cells, but it augmented a monoamine transporter-dependent enhancement of phenethylamine activation of TA1 in TA1-DAT and TA1-NET cells, but not in TA1-SERT cells. The present data provide compelling evidence that modafinil occupies the DAT and NET in living brain of rhesus monkeys and raise the possibility that modafinil affects wakefulness by interacting with catecholamine transporters in brain.

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ABBREVIATIONS: DAT, dopamine transporter; NET, norepinephrine transporter; PET, positron emission tomography; CFT (WIN 35,428), 2β-carbomethoxy-3β-(4-fluorophenyl)tropane; MeNER, (S,S)-2-[(2-methoxyphenox)benzyl)morpholine; PEA, phenethylamine; TA1, trace amine receptor 1; HEK, human embryonic kidney; SERT, serotonin transporter; DA, dopamine; CRE, cAMP response element; RLU, relative light unit; TAR1, rhesus monkey trace amine receptor 1.
(Lafon, 1979), all of which are characteristics of hyperdopaminergic activity. Thereafter, several investigators questioned the contribution of dopamine neurotransmission to modafinil pharmacology, on the basis of overlapping, but not identical behavioral and neurochemical responses of modafinil compared with amphetamine, a psychomotor stimulant with robust dopaminergic effects (Duteil et al., 1990; Akaoka et al., 1991; Simon et al., 1995; Lin et al., 1996; Ferraro et al., 1997a,b; Engber et al., 1998; Engle, 1998). Modafinil was a weak inhibitor of the DAT (>1 μM affinity) and displayed no affinity for dopamine receptor subtypes (Mignot et al., 1994; Bryan Roth, personal communication). Enhancement of glutamate release and inhibition of GABA release in various brain regions were proposed as alternative modes of action (Ferraro et al., 1997a,b, 1999).

Notwithstanding these important observations, accumulating behavioral and biochemical evidence converges on dopaminergic and noradrenergic mechanisms as plausible contributors to modafinil pharmacology (Wisor et al., 2001; Boutrel and Koob, 2004; Wisor and Eriksson, 2005). Modafinil engenders behavioral effects consistent with enhanced dopamine transmission, because it increases motor activity in normal or parkinsonian monkeys (Jenner et al., 2000; van Vliet et al., 2006), partially generalizes to a cocaine-like discriminative stimulus in rodents, and serves as a reinforcer at high doses in monkeys or human subjects (Gold and Balster, 1996; but see Deroche-Gamonet et al., 2002; Stoops et al., 2005). Modafinil also increases striatal dopamine efflux as effectively asamphetamine in dog brain, with dose ratios comparable with clinical dose ratios. Significantly, the wake-promoting effects of modafinil are abolished in DAT null mutant mice (Wisor et al., 2001).

The rapid expansion of new therapeutic indications for modafinil, in the face of unexplained mechanisms, creates an exigency to clarify modafinil pharmacology. On the basis of modafinil-induced dopamine efflux and the DAT dependence for the wake-promoting effects of the drug (Wisor et al., 2001), we postulate that modafinil modulation of the DAT (or NET) contributes to enhanced wakefulness and therapeutic benefit. This hypothesis is predicated on whether modafinil occupies the DAT or NET in living brain, an objective that, to our knowledge, has not been addressed. We used PET imaging of the DAT ([11C]CFT) and of the NET with [11C]MeNER, our knowledge, has not been addressed. We used PET imaging

### Materials and Methods

**PET Imaging of the Dopamine and Norepinephrine Transporter.** Adult female (n = 4) and male (n = 1) rhesus monkeys (*Macaca mulatta*) were used for PET imaging procedures. Animal care and treatment were supervised by veterinarians under the guidelines and in accordance with Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy Press, Washington, DC, 1996). The animal care protocol was approved by the Harvard Animal Care Committee and was in compliance with the Harvard Medical School animal management program, an institution accredited by the American Association for the Accreditation of Laboratory Animal Care.

PET imaging of the DAT in caudate putamen was used to determine DAT occupancy by modafinil. DAT was quantified with the selective DAT probe [11C]CFT (Kauffman and Madras, 1991; Hantraye et al., 1992; Morris et al., 1996; Madras et al., 1998; Saka et al., 2004). PET imaging was conducted with monkeys initially anesthetized with ketamine/xylazine (15.0/1.5 mg/kg) and then maintained under general anesthesia with halothane. Monkeys were positioned on the imaging bed of a PC 4096 PET camera (GE/Scanditron AB, Uppsala, Sweden). A stereotactic head-holder was used for head immobilization. CFT was demethylated in the C-2 position, and [14C]methyl was inserted by the methyl iodide reaction. After stabilization in the PET camera, ~10 mCi of [14C]CFT (specific activity >1500 mCi/μmol) was injected through the venous catheter and sequential images were acquired in 15-s time frames for the first 2 min and in 1-min frames for 58 min. DAT or NET occupancy was measured by administering modafinil i.v. 1 h after baseline image acquisition was completed and then reintroducing radioligand via an i.v. indwelling catheter 1 h later. Parallel studies were conducted with the NET probe [11C]MeNER. At the conclusion of each imaging study, the emission and transmission images were reconstructed using a conventional filtered back-projection algorithm to an in-plane resolution of 6-mm full-width half-maximum. All projection data were corrected for nonuniformity of detector response, dead time, random coincidences, and scattered radiation. A sum image was generated by adding all of the frames from frame 10 to the end of the study. Regions of interest were drawn on the summed image in the coronal projection as follows: one 4-pixel region was drawn on each caudate putamen on the slice of maximal intensity. For cerebellum, three 4-pixel regions were drawn at various levels on cerebellar slices. Time-activity data were produced using the regions of interest on all time frames of the PET data. The same set of regions of interest was used to analyze each scan for an individual subject on the same day. When necessary, new regions were drawn to compensate for repositioning. Binding potential was calculated by published methods. Parallel experiments were conducted with the PET ligand [11C]MeNER (Schou et al., 2004; Ghose et al., 2005) to image the NET in the thalamus and monitor modafinil occupancy.
Dopamine, Norepinephrine, and Serotonin Transporter Assays. The generation of stable human DAT-, NET-, and SERT-transfected HEK293 cells has been described previously (Goulet et al., 2001; Yatin et al., 2001; Verrico et al., 2005). Assays to measure drug inhibition of [3H]monoamine transport were performed to determine IC₅₀ values, as described previously (Miller et al., 2001; Verrico et al., 2005). Cells were grown to 70 to 85% confluence in Biocoat 24-well tissue culture plates coated with polylysine. The medium was removed by aspiration, and cells were washed with ice-cold assay buffer: Tris–HEPES, pH 7.4, at 25°C containing 4 mM Tris base, 8.5 mM HEPES, 120 mM NaCl, 5.4 mM KCl, 1.2 mM CaCl₂, 1.2 mM MgSO₄, 10 mM glucose, 100 μM ascorbic acid, and 100 μM tropolone. Cells were harvested by pipetting the medium for cell detachment and resuspended in the assay buffer at approximately 3 x 10⁵/ml cells. Each assay tube contained 1.5 x 10⁵ cells, [3H]DA, [3H]norepinephrine, or [3H]5-hydroxytryptamine and various concentrations of unlabeled monoamine to achieve a total of 0.4 ml of solution. Uptake activities were initiated by transferring the tubes from ice to 37°C water bath and terminated 3 or 10 min later by transferring the tubes to ice. Each experiment was performed in triplicate and repeated three to six times. All experiments were conducted in darkness. Nonspecific monoamine transport was measured with 10 μM mazindol for DAT, 10 μM nisoxetine for NET, or 10 μM fluoxetine for SERT. In competition experiments, 0.2 ml of drug was added for a 10-min preincubation period. Monoamine transport was initiated by the addition of [3H]monoamine combined with various concentrations of unlabeled monoamine to achieve an appropriate specific activity (Sigma-Aldrich, St. Louis, MO). The assay was terminated by aspiration. The plate was placed on ice and rinsed three times with cold (4°C) assay buffer. After the final wash was aspirated, 0.5 ml of 10% SDS was added to each well. After 20 min of gentle shaking, 0.25 ml was removed from each well, and radioactivity was measured for 5 min in vials containing 4 ml of ReadySafe scintillation fluid. IC₅₀ values were obtained by nonlinear curve fitting using Prism version 3.0 (GraphPad Software Inc., San Diego, CA).

Rhesus TA1 Activity, as Determined by Dual-Luciferase Reporter Assay. Stable DAT, NET, and SERT cells were placed in 48-well plates 2 days before transfection. Transfections were performed on 70 to 80% confluent cells as described previously (Miller et al., 2005; Xie et al., 2005). In total, three vectors were cotransfected: 1) a CAMP-response element (CRE)-driven firefly luciferase reporter construct, pTLNC121-3 (from Dr. Walter Borne, University of Zurich, Zurich, Switzerland), which contains a 21×CRE cassette positioned upstream from a TATA box minimal promoter element, as a highly sensitive and quantitative reporter of total cAMP accumulation; 2) an optimized Renilla luciferase construct, pGL4.73 (Promega, Madison, WI), which is unresponsive to increases in cellular cAMP (G. M. Miller, unpublished data), to control for transfection efficiency and experimental variability; and 3) either a TA1 expression vector (Miller et al., 2005) or pDNA3.1(+) (Invitrogen, Carlsbad, CA) to control for total DNA amounts used in transfections. Transfections into different cell lines were performed simultaneously for each experiment. Dual-luciferase assay (Promega) was performed according to the manufacturer’s protocol. Cells were incubated with transfection solution for 12 h and then exposed to drugs at different concentrations for 18 h at incubation conditions. Monoamine transporter inhibitors, when used, were applied to the cells at 15 min before the drug treatment. Cell lysates were prepared by adding 100 μl of lysis buffer into each well and shaking on a rotator platform for 30 min at 25°C. Then, 20 μl of each cell lysate was transferred into a 96-well microplate for determination of luciferase concentration. Luciferase assay substrate reagents (25 μl) were injected into each sample well, and after a 2-s delay, luciferase concentration was measured as relative light units (RLUs) for 12 s at Wallac 1420 multilabel counter-Victor 3V (PerkinElmer Life and Analytical Sciences, Shelton, CT). The experiments were performed three times in triplicate for each concentration of the drug treatment. Percentage of RLU increase was calculated from the ratio of firefly to Renilla luciferase in cell lysates. To assess the effect of monoamine transporter blockers on TA1 in cells that coexpress a monoamine transporter, we defined the enhancement of TA1 activation as the percentage of increase of RLU in TA1-transfected DAT, NET, or SERT cells subtracted by the percentage of increase of RLU in TA1-transfected HEK293 cells.

Drugs. [3H]Dopamine, [3H]norepinephrine, and [3H]serotonin were purchased from PerkinElmer Life and Analytical Sciences (Wellesley, MA). Modafinil and PEA were purchased from Sigma-Aldrich.

Results

PET Imaging: DAT and NET Occupancy by Modafinil. To investigate whether clinically relevant doses of modafinil accumulate at the DAT, we labeled the DAT with the PET imaging probe [11C]CFT or WIN 55,428 (Madras et al., 1989; Morris et al., 1996). [11C]MeNER was used to label the NET (Schou et al., 2004). In a double-injection study, rhesus monkeys were scanned to obtain baseline measures of DAT or NET binding potential. After decay of [11C]CFT or [11C]MeNER, monkeys were injected i.v. with 2, 5, or 8 mg/kg modafinil, and PET imaging was repeated 1 h later (Table 1). DAT occupancy was insignificant with 2 mg/kg modafinil (n = 1), but 5 mg/kg modafinil occupied 35 ± 11.9% of DAT sites (n = 4), and 8 mg/kg modafinil occupied 53 ± 3% (n = 3) of DAT sites in striatum (Figs. 1, left, and 2; Table 1). Modafinil (8 mg/kg) occupancy of the DAT (Fig. 2) or the NET (Fig. 3) was observable within 60 min after administration. To verify that modafinil occupancy occurred regardless of the DAT probe, a single parallel experiment was performed with the DAT probe [11C]altropane (Fischman et al., 2001). Again, 8 mg/kg modafinil occupied 67% of DAT sites, indicating that DAT occupancy by modafinil was not an artifact of the PET probe. For comparative purposes, a parallel study was conducted with methylphenidate, again at a clinically relevant dose (0.3 mg/kg). Methylphenidate occupied comparable levels of DAT at a dose of 0.3 mg/kg (Table 1).

The NET also accumulated modafinil (Figs. 1, right, and 3), with a 5-mg/kg dose occupying 16 ± 7.6% of NET sites (n =

| Drug     | Dose | % DAT Occupancy (|11C|CFT) | % NET Occupancy (|11C|MeNER) |
|----------|------|-----------------|-----------------|
| Modafinil| 2    | 6 ± 6 (2)       | 16 ± 7.8 (6)    |
| Modafinil| 5    | 35 ± 12 (4)     | 44 ± 12 (2)     |
| Modafinil| 8    | 54 ± 2.96 (3)   |                 |
| Modafinil| 8    | [11C]Altropane: 67 (1) |              |
| Methylphenidate | 0.3 | 51 ± 10 (2)    |                |
i.v. and 1 h later, \([11C]\)MeNER was injected. \([11C]\)MeNER accumulation was significantly lower compared with baseline levels of accumulation (left). Data are summarized in Table 1 and Fig. 1. Images were color-transformed to display occupancy of the DAT with \([11C]\)CFT (five rhesus monkeys) and of the NET with \([11C]\)MeNER (four rhesus monkeys). Occupancy was calculated as described under Materials and Methods. For each dose of modafinil, PET imaging was conducted in two to five different monkeys, with each point representing the mean \(\pm\) S.E.M. of two to six independent experiments, conducted on different days. For representative images, please see Figs. 2 and 3. For 8 mg/kg modafinil, one data set is shown for %NET occupancy.

**Fig. 1.** Modafinil occupancy of the DAT in monkey striatum (left) or NET in monkey thalamus (right). PET imaging of the DAT was conducted with \([11C]\)CFT (five rhesus monkeys) and of the NET with \([11C]\)MeNER (four rhesus monkeys). Occupancy was calculated as described under Materials and Methods. For each dose of modafinil, PET imaging was conducted in two to five different monkeys, with each point representing the mean \(\pm\) S.E.M. of two to six independent experiments, conducted on different days. For representative images, please see Figs. 2 and 3. For 8 mg/kg modafinil, one data set is shown for %NET occupancy.

**Fig. 2.** Modafinil (8 mg/kg) occupancy by the DAT in caudate putamen, as detected by PET imaging of the DAT with \([11C]\)CFT. Left, an adult rhesus monkey was injected with \([11C]\)CFT and scanned over 60 min to develop baseline measures of DAT binding potential in the caudate putamen. Images were color-transformed to display occupancy of the DAT with \([11C]\)CFT, with highest levels detected in caudate putamen (white-red), as designated by the arrow, and lowest levels in blue-purple. Regions of interest are drawn over the caudate putamen. Right, after decay of \([11C]\)CFT radioactivity, modafinil was injected i.v., and \([11C]\)CFT was injected again 1 h later. \([11C]\)CFT accumulation was significantly lower compared with baseline levels of accumulation (left). Data are summarized in Table 1 and Fig. 1.

**Fig. 3.** Modafinil (8 mg/kg) occupancy by the NET in thalamus, as detected by PET imaging of the NET with \([11C]\)MeNER. Left, an adult rhesus monkey was injected with \([11C]\)MeNER and scanned over 60 min to develop baseline measures of NET binding potential in the thalamus. Images were color-transformed to display occupancy of the NET by \([11C]\)MeNER, with high levels detected in the thalamus (white-red), as designated by the arrow, and lowest levels in blue-purple. Regions of interest are drawn over the thalamus. Right, after decay of \([11C]\)MeNER radioactivity, modafinil was injected i.v. and 1 h later. \([11C]\)MeNER was injected. \([11C]\)MeNER accumulation was significantly lower compared with baseline levels of accumulation. Data are summarized in Table 1 and Fig. 1.
in intracellular localization of TA1 in vitro, because TA1 activation by PEA is dose-dependent and is robustly enhanced by either DAT, NET, or SERT coexpression in vitro (Fig. 7). The enhancement of PEA activation of TA1 in the presence of DAT, NET, or SERT is blocked by pretreatment with the transporter inhibitors methylphenidate, desipramine, and citalopram, respectively, at a dose of 10 μM (Fig. 7). In the absence of monoamine transporters, modafinil (10⁻⁹–10⁻⁴ mol/l) had no effect on PEA activation of TA1 (Fig. 8A, left top). We tested a single dose of modafinil, 10 μM, and found that it was able to augment the enhancement of PEA activation of TA1 in the presence of DAT (Fig. 8B, right top) or NET (Fig. 8C, left bottom), but there was no augmentation of the enhancement of PEA activation of TA1 in the presence of SERT.

Discussion

Modafinil is widely used to treat narcolepsy (Banerjee et al., 2004), and it is undergoing clinical trials as a therapeutic for a wide range of psychiatric, neurological, and medical illnesses. These include cocaine addiction (Dackis and O’Brien, 2003; Voci and Ling, 2005), Parkinson’s disease (Serrano and Garcia-Borreguero, 2004), attention deficit hyperactivity disorder (Biederman et al., 2005), depression (Price and Taylor, 2005), cancer, and multiple sclerosis-related fatigue (Morrow et al., 2005) and opioid-induced sedation in chronic pain (Reissig and Rybarczyk, 2005). The rapid expansion of these presumptive clinical indications for modafinil is empirically driven, because no broad consensus exists on the underlying mechanisms of modafinil pharmacology. We now provide compelling evidence that modafinil occupies the DAT and NET in living brain of rhesus monkeys at doses that are presumably clinically relevant. Occupancy was comparable with DAT occupancy by the attention deficit hyperactivity disorder medication methylphenidate, also administered at a therapeutic dose. The clinical significance of this discovery is supported by in vitro data, because modafinil blocked 50% of DAT-mediated dopamine transport in vitro at a lower concentration than the plasma concentrations of modafinil estimated in human subjects within 2 h after a modest dose (200 mg) of the drug (Wong et al., 1999). Accordingly, clinically relevant doses (400–600 mg) of modafinil conceivably achieve brain concentrations sufficient to bind to DAT and NET, notwithstanding the caveats of 1) extrapolating modafinil plasma levels to brain extracellular concentrations, 2) extrapolating in vitro to in vivo transporter affinities, 3) potential differences in the pharmacokinetic and metabolic properties of modafinil in humans and nonhuman primates, and 4) a similarity in plasma levels that

**Table 2**

<table>
<thead>
<tr>
<th>Drug or Compound (n)</th>
<th>DAT IC₅₀</th>
<th>NET IC₅₀</th>
<th>SERT IC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modafinil*</td>
<td>6390 ± 2050 (3)</td>
<td>35,600 ± 5300 (6)</td>
<td>170,000 ± 30,000 (3)</td>
</tr>
<tr>
<td>Methylphenidate</td>
<td>25.4 ± 3.2 (7)</td>
<td>26.5 ± 3.56 (4)</td>
<td>N.D.</td>
</tr>
<tr>
<td>Benztropine</td>
<td>213 ± 50 (5)</td>
<td>667 ± 76 (3)</td>
<td>N.D.</td>
</tr>
<tr>
<td>Bupropion</td>
<td>1088 ± 222 (3)</td>
<td>N.D.</td>
<td>21,680 ± 4831 (3)</td>
</tr>
</tbody>
</table>

N.D., not done.

* Modafinil affinity in radioligand binding assay for the DAT; [³H]CFT IC₅₀ = 4360 nM.
results from i.v. modafinil in rhesus monkeys compared with oral modafinil in humans. If our data are confirmed in human subjects by PET imaging, we surmise that modafinil is a modulator of DAT and/or NET function in various brain regions, a process that conceivably contributes to enhanced wakefulness and possibly other therapeutic effects of the drug.

Although initial observations of modafinil pharmacology were consistent with drug-induced enhancement of dopamine activity, the contribution of dopamine neurotransmission to modafinil pharmacology has been repeatedly questioned, on the basis of nuances of neurochemical or behavioral data and in vitro DAT affinity. In particular, dopaminergic contributions were mitigated by evidence of contrasting amphetamine and modafinil pharmacology, brain activation patterns, and the failure of dopamine receptor antagonists to attenuate modafinil-mediated behavioral effects (Duteil et al., 1990; Akaoka et al., 1991; Mignot et al., 1994; Simon et al., 1995; Lin et al., 1996; Ferraro et al., 1997a; Engber et al., 1998). Although these differences may be attributable to contrasting doses, potencies, and the spectrum of molecular targets and affinities for various receptors and transporters in brain, they do not exclude dopaminergic mechanisms for both drugs.

A functional interaction between DAT and modafinil has been discounted, because of low modafinil affinity for DAT (Mignot et al., 1994). This is an insufficient reason to disregard the DAT, because relatively high doses of modafinil ranging from 200 to 600 mg (e.g., Schwartz et al., 2005) are administered clinically. The doses we used to detect DAT occupancy were 2 to 8 times lower than oral doses used to promote wakefulness in monkeys (Hermant et al., 1991). At a 200-mg dose, initial plasma modafinil levels range from 15 to 19 μM (Wong et al., 1999), a concentration 3 times higher than the 6 μM modafinil concentration needed to inhibit 50% of DAT sites in vitro. Other DAT modulators (e.g., amphetamine or methylphenidate) have much higher DAT affinity, and accordingly, are given at far lower doses (approximately 5–20 mg).

Our finding of modafinil occupancy of striatal DAT sites provides evidence that DAT modulation by modafinil may contribute to modafinil pharmacology. This hypothesis is
in vitro. Other factors, in addition to in vitro affinity, conceivably dictate the extent of DAT or NET occupancy, including ligand dissociation rates, relative affinities of the two PET probes, and relative DAT or NET densities in these brain regions. Furthermore, levels of endogenous substrates (neurotransmitters and modulators) in various brain regions are likely to affect modafinil interaction with DAT and NET. Modafinil is a relatively weak inhibitor of catecholamine transporters, and its capacity to block transporter function will be sensitive to local concentrations of competing endogenous neurotransmitters, to a greater extent than other DAT or NET inhibitors, with affinities 10 to 100 times higher (e.g., cocaine or methylphenidate). Although we did not monitor SERT occupancy, given the exceedingly low affinity of modafinil for the SERT, it is unlikely that therapeutic doses of the drug will interact with SERT to affect SERT function directly, but indirect effects cannot be ruled out.

Based on the in vivo and in vitro data, it is reasonable to surmise that modafinil could elevate dopamine and possibly norepinephrine, and perhaps also trace amine levels in select brain regions, and thereby indirectly activate dopamine, norepinephrine, and/or trace amine receptor subtypes. Elevation of dopamine levels has not conventionally been implicated in sleep-wake regulation, but the present study and that of Wisor et al. (2001) implicate the DAT as a potential mediator of modafinil-induced wakefulness. Potent DAT inhibitors, such as difluoropine or indatraline, promoted sleep fragmentation and increased nighttime wakefulness (Madras et al., 2006). The role of norepinephrine is less certain, because chemical ablation of noradrenergic neurons did not block modafinil-induced wakefulness (Wisor and Eriksson, 2005). It has been postulated that modafinil increases wakefulness by promoting glutamate release and inhibiting GABA release (Duteil et al., 1990; Ferraro et al., 1997a,b, 1999; Wisor et al., 2001). In parallel with modafinil, the DAT inhibitor cocaine also promotes glutamate release and attenuates GABA release in select brain regions (Cameron and Williams, 1994; Reid et al., 1997).

We found that the monoamine transporter inhibitors methylphenidate, desipramine, and citalopram at 10 μM almost entirely block DAT-, NET-, or SERT-dependent enhancement of TA1 activation by PEA. The enhanced response of TA1 to monoamines observed with the CRE-luc assay may be due to the ability of monoamine transporters to deliver agonists intracellularly, where TA1 receptors are observed to be sequestered in vitro (Bunzow et al., 2001; Miller et al., 2005). However, it may also be that during the long period of agonist treatment (18 h) required to maximize the signal-to-noise ratio in the CRE-luc assay, TA1 receptors may traffic to the membrane, and that this trafficking is somehow facilitated by transporter function, although this has yet to be investigated. We also tested modafinil at the same dose (10 μM), which we show does not directly activate TA1. But unlike the other inhibitors, which have 15 to 100 times higher potency, 10 μM modafinil is near its IC_{50} value for the DAT. At this dose, modafinil augmented the enhancement of TA1 activity by PEA in DAT- and NET-expressing cells, but not SERT-expressing cells. This finding gives an initial indication that modafinil could affect, albeit indirectly, PEA activation of the TA1 receptor in dopamine and norepinephrine neurons. TA1 receptor mRNA is present in the major monoaminergic cell groups (Borowsky et al., 2001), and we have

![Fig. 7. Dose-response effects of PEA in TA1-transfected HEK cells (TA1) compared with TA1-transfected DAT (DAT-TA1), NET (NET-TA1), or SERT (SERT-TA1) cells. Pretreatment of DAT-TA1, NET-TA1, or SERT-TA1 cells with 10 μM methylphenidate, 10 μM desipramine, or 10 μM citalopram, respectively, reduces the transporter-dependent enhancement of TA1 activation.](image_url)
used double-label DAT and TA1 immunocytochemistry to demonstrate that a subset of dopamine neurons express TA1 receptors in both rhesus monkey and mouse substantia nigra (Xie et al., 2005). Regardless, the augmentation of enhancement of PEA activation of TA1 receptors by modafinil in DAT- and NET-expressing cells, but not SERT cells, further supports the hypothesis that modafinil interacts with the DAT and NET.

The interaction of modafinil with the DAT may account for some of its preliminary positive clinical results in cocaine addicts (Voci and Ling, 2005). Although the underlying mechanisms are unknown for this indication, the low DAT affinity of modafinil may be advantageous as a cocaine medication. In this regard, it is likely that extracellular dopamine inunction that ensues from potent DAT inhibitor may lead to undesirable receptor adaptations, arising from the inability of pre- or postsynaptic receptors to recycle appropriately, particularly if dopamine levels achieve a concentration that is likely to bind to dopamine receptors in the low-affinity state.

In summary, the present data raise the possibility that modafinil affects wakefulness by interacting with catecholamine transporters in brain. Although modafinil is likely to mediate effects via several mechanisms, the present study supports the view that the DAT and NET warrant further scrutiny as an important molecular target for this intriguing drug.

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