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Alpha-ethyltryptamines as dual dopamine–serotonin releasers

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ABSTRACT

The dopamine (DA), serotonin (5-HT), and norepinephrine (NE) transporter releasing activity and serotonin-2A (5-HT_{2A}) receptor agonist activity of a series of substituted tryptamines are reported. Three compounds, **7b**, (+)-**7d** and **7f**, were found to be potent dual DA/5-HT releasers and were >10-fold less potent as NE releasers. Additionally, these compounds had different activity profiles at the 5-HT_{2A} receptor. The unique combination of dual DA/5-HT releasing activity and 5-HT_{2A} receptor activity suggests that these compounds could represent a new class of neurotransmitter releasers with therapeutic potential.

Biogenic amine neurotransmitters—dopamine (DA), serotonin (5-HT) and norepinephrine (NE)—are critically involved in the pathogenesis and treatment of various psychiatric disorders.[1] The three biogenic amine transporters (BATs) are principal sites of action for cocaine and amphetamines. It is useful to divide BAT ligands into two categories based on their molecular mechanism of action: uptake inhibitors and substrates (for a review[2]). The uptake inhibitor cocaine binds to BATs and inhibits neurotransmitter uptake, leading to increases in synaptic transmitter levels. The cocaine-induced rise in extracellular neurotransmitters is dependent upon impulse-driven exocytosis and is sensitive to negative feedback loops. Amphetamine-like compounds are BAT substrates that release transmitters via carrier-mediated exchange, a process that is not dependent on impulse-driven exocytosis and is not sensitive to negative feedback loops. As a result, administration of BAT substrates tends to produce larger elevations in extracellular neurotransmitters than administration of uptake inhibitors.[3]

A major goal of our laboratory has been the development of medications that ameliorate the symptoms of stimulant withdrawal and thereby facilitate abstinence. Although many strategies for addressing this problem are possible (for recent reviews[4–7]) we decided to first focus our efforts on dual selective DA and 5-HT releasing agents for several reasons. First, preliminary clinical observations indicated that co-administration of phentermine (an amphetamine-like DA-releaser) and fenfluramine (a 5-HT releaser) showed promise in treating cocaine and alcohol dependence.[8] Second, administration of DA and 5-HT releasing agents alone or together decrease drug-seeking behavior.[9–12] Third, based on literature suggesting cocaine withdrawal causes dysfunction in both the DA and 5-HT systems (see above), it seemed logical to develop a medication that normalizes deficits in both neurotransmitter systems, rather than just one. Fourth, a series of investigations indicated that concurrent 'global' elevation of brain extracellular 5-HT attenuates the psychomotor stimulant and rewarding effects that result from elevation of extracellular DA, without actually decreasing extracellular DA.[13,14] Similar findings with DA/5-HT uptake inhibitors broadly reinforce the validity of our findings.[15] Finally, we believed that candidate medications for stimulant addictions should target the same 'receptors' as the primary drug of abuse. This strategy, known as 'agonist therapy', is a proven approach for treating substance use disorders as exemplified by the efficacious treatments for cigarette smoking (e.g., the nicotine patch) and opioid dependence (e.g., methadone and buprenorphine).[16] In the context of stimulant dependence, the target 'receptors' of interest are BATs.

It is now well established that DA releasing agents, such as D-amphetamine, decrease cocaine self-administration behavior in animals[10,11,17,18] and that D-amphetamine and methamphetamine are two of the few medications to show promise as a treatment agent for stimulant addiction in controlled clinical trials.[16,19,20] These data provide support for using the agonist substitution approach for developing potential medications for the treatment of stimulant dependence. However, a significant limitation of this approach is the abuse liability of DA releasing agents.[21] As noted

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above, concurrent elevation of extracellular 5-HT reduces D-amphetamine-induced stimulant and rewarding effects without reducing D-amphetamine increases in extracellular DA, and without altering the ability of D-amphetamine to decrease cocaine self-administration.[3] Seen from this perspective, concurrent elevation of extracellular 5-HT can reduce the abuse liability of D-amphetamine-like agents without altering the therapeutic effect, which is mediated by the increased release of DA.

This type of profile was observed with PAL-287 (compound **1**, 1-naphthyl-2-aminopropane), a universal BAT releaser evaluated as our original proof of principle.[22] PAL-287 was found to induce transporter mediated release of DA, 5-HT, and NE with EC_{50} values of 12.6 nM, 3.4 nM, and 11.1 nM, respectively. In vivo microdialysis in rats demonstrated that PAL-287 (1-3 mg/kg, iv) increased extracellular DA and 5-HT with the effects on 5-HT being somewhat greater. PAL-287 induced substantially less locomotor stimulation than (+)-amphetamine, a drug that increases only extracellular DA. PAL-287 did not produce serotonergic 'neurotoxicity' and had little or no reinforcing properties in rhesus monkeys trained to self-administer cocaine. Yet, PAL-287 produced a dose-dependent decrease in responding for cocaine when infused at a dose of 1.0 mg/kg/h. Although the addition of 5-HT releasing activity to a DA releaser decreases behavioral selectivity (less separation between cocaine vs food responding behavior),[17,18] the clinical implications of these findings are not clear, in view of the fact that patients treated with various serotonergic medications do not appear to suffer from non-specific disruptions of behaviors related to the reward pathways. Our results with PAL-287 support the hypothesis that a non-amphetamine substrate at DATs and SERTs will release DA and 5-HT from neurons in vivo, be minimally reinforcing, and also suppress ongoing cocaine self-administration.

The inclusion of NE release in a pharmacotherapy for the treatment of substance abuse is less clear. Dual DA/NE releasing agents, such as amphetamine and phentermine, continue to be safely used in the clinic.[3] NE reuptake inhibitors, either administered alone or as dual NE/5-HT reuptake inhibitors, have been safely used as antidepressants.[23] Despite the favorable safety record associated with using NE reuptake inhibitors and releasing agents in the clinic, some studies suggest an undesirable cardiotoxic component may exist with NE elevation. Ephedrine and analogs, which have been shown to be primarily NE releasing agents,[24] have been linked to adverse cardiovascular events including sudden cardiac death.[25] Furthermore, NE has been shown to increase oxidative stress on the cardiac cells of rats by auto-oxidation,[26] and myocardial ischemia has been shown to be enhanced by NE elevation mediated by superoxide anion radicals.[27] Because of these issues, one goal of our releaser medications development program has been to identify compounds that induce DA and 5-HT release without having NE releasing properties in order to avoid cardiotoxicity complications. We hypothesized that such a molecule would maximize therapeutic effects while minimizing potential toxicities. Herein, we report the identification of the first class of dual DA/5-HT releasers with at least 10-fold less releasing activity at the NE transporter (NET) over the DAT and SERT.

Twenty-five tryptamines were studied in BAT release and uptake inhibition assays as well as in a 5-HT 2A ($5\text{-}HT_{2A}$) receptor assay since tryptamines are known to interact with the $5\text{-}HT_{2A}$ receptor and $5HT_{2A}$ agonists are thought to be hallucinogenic.[28] The transporter activity of all compounds was assessed using the previously described protocol for identifying releasers and uptake inhibitors, using synaptosomes generated from rat brain homogenate.[29,30] Compounds were binned as releasing substrates or uptake inhibitors by assessment in both synaptosomal release assays and uptake inhibition assays. Once the functional activity of a compound was determined, dose response curves were completed for the binned activity. $5\text{-}HT_{2A}$ receptor agonist activity was measured in an in vitro calcium mobilization assay using cells over-expressing the $5\text{-}HT_{2A}$ receptor. All of the compounds were either purchased or synthesized in our laboratory. Tryptamines **4a**, **4b**, **4d**, **4g**, **4h**, **4j**, **4l**, and **4m** were purchased commercially. Tryptamines **4c**, **4e**, **4f**, **4i**, **4k**, **4n**, **4o**, **4p** and **4q** were synthesized by reacting the requisite substituted indole with oxalyl chloride followed by reaction with either ammonia or ethylamine in dioxane and subsequent reduction with lithium aluminum hydride (Scheme 1).[31] The racemic α-alkyl tryptamines **7a**, **7b**, **7c**, **7d**, **7e**, and **7f** were synthesized by nitro olefin formation followed by lithium ammonium hydride reduction (Scheme 2).[32] The optically active tryptamines (−)-**7d** and (+)-**7d** were synthesized by reacting the N-protected 3-bromoindole with *n*-butyllithium, then adding (+)- or (−)-propylene oxide to form optically active alcohols **8** (Scheme 3, shown for (+)-**7d**).[33] The secondary alcohol was then converted to the amine by forming the tosylates **9** followed by azide displacement, azide reduction, and finally deprotection to form the optically active tryptamines (−)-**7d** and (+)-**7d**.

All but one compound, 4-methoxytryptamine (**4f**) were 5-HT-releasing substrates (Table 1). Twenty-two of the twenty-four 5-HT releasers were very potent, with EC_{50} values under 140 nM. The most potent 5-HT releaser was 7-chlorotryptamine (**4p**) with an EC_{50} value of 8 nM. This is comparable to PAL-287, with an EC_{50} value of 4 nM. It was also one of the most selective 5-HT releasers, as were all the 7-substituted analogs. Tryptamines are not normally known to possess strong stimulant activity, yet ten of the compounds had EC_{50} values for DA release of less than 165 nM. All five fluoro derivatives tested, 4-fluorotryptamine (**4e**), 5-fluorotryptamine (**4j**), 6-fluorotryptamine (**4l**), 5-fluoro-α-methyltryptamine (**4c**), and 5-fluoro-α-ethyltryptamine (**7f**) were potent DA releasers with EC_{50} values of 106 nM, 82.3 nM, 104 nM, 31.8 nM and 150 nM, respectively. The other five compounds were tryptamine (**4a**), 6-methoxytryptamine (**4m**), α-methyltryptamine (**7a**), 5-chloro-α-methyltryptamine (**7b**) and (*S*)-α-ethyltryptamine ((+)-**7d**).

Surprisingly, most of the compounds were not active as NE releasers. To date we have screened over 1000 small arylalkylamines for activity at all three biogenic amine transporters and normally NE release parallels DA release, and is usually slightly more potent. However in the case of the tryptamines, the NE releasing activity of the DA releasers was weaker in almost every case, and often by at least an order of magnitude (>10-fold). This activity profile implies that these compounds do not interact with the NET as well as they interact with the DAT. The NE release potencies for (*S*)-α-ethyltryptamine ((+)-**7d**), 4-fluorotryptamine (**4e**), 6-fluorotryptamine (**4l**), 5-fluoro-α-ethyltryptamine (**7f**), and 5-chloro-α-methyltryptamine (**7b**) were 10-, 11-, 30-, and 64-, and 36-fold weaker than their DA release potencies. In addition, several compounds including 5-chlorotryptamine (**4h**), 5-bromotryptamine (**4i**), and 6-methyltryptamine (**4n**) were inactive as NE releasers at 10 μM but maintained reasonable potencies as DA releasers (EC_{50} values < 500 nM). In addition, these compounds were found to have fairly robust 5-HT releasing potencies with EC_{50} values from 12.9 nM to 75 nM making them the first dual selective DA/5-HT releasers discovered in our project.

From a structure activity relationship perspective, N-methylation did not improve the selectivity or potency compared to tryptamine (**4a** vs **4b**). N-ethylation caused the scaffold to lose its DA releasing activity (**4a** vs **4c**). Alkylation of the 1-position (nitrogen on the indole ring, **4d**) also caused the scaffold to lose its DA releasing activity. N-alkylation at either the side chain nitrogen or the indole ring does not appear to be a viable approach for optimizing dual DA/5-HT releasing activity. α-Alkylation of tryptamine to form α-methyltryptamine (**7a**), similar to the difference between phenethylamine and amphetamine, caused the DA releasing potency to improve 2-fold and the NE-releasing potency to