MINIREVIEW

Trace Amine-Associated Receptors as Emerging Therapeutic Targets

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ABSTRACT

Endogenous trace amines (TAs) of unknown biological function are structurally related to classic monoaminergic neurotransmitters and found at low concentrations in the mammalian brain. Their recently discovered group of G protein-coupled receptors, trace amine-associated receptors (TAARs), may represent putative targets not only for trace and other amines but also for a variety of monoaminergic compounds, including amphetamines and monoamine metabolites. The trace amine-associated receptor 1 (TAAR1), which is in part associated with the monoaminergic neuronal circuitry controlling various functions, including movement, is the best characterized of the class, although little is known about its regulation and function. Here we review the pharmacology and biochemical properties of the TAAR1 and its physiological functions as revealed in studies involving knockout mice lacking this receptor. Potential therapeutic applications of future selective TAAR1 agonists and antagonists are also discussed. Although understanding of biology and functions mediated by other TAARs is still in its infancy, it is expected that further characterization of the functional roles and biochemical properties of TAARs and identification of endogenous and exogenous ligands will eventually promote these receptors as an attractive class of targets to correct monoaminergic processes that could be dysfunctional in a host of disorders of brain and periphery.

The classic monoaminergic neurotransmitters (dopamine, serotonin, norepinephrine, epinephrine, and histamine) play critical roles in various physiological processes in vertebrates and have become principal targets for many pharmacological approaches to treat human disorders. Among the various processes involved in monoaminergic homeostasis and neurotransmission, such as synthesis, packaging into vesicles, release, reuptake, and metabolism, the targeting of specific monoaminergic receptor proteins has proven to be the most effective approach to correct dysfunctions in many pathological conditions, including Parkinson’s disease and schizophrenia. These receptors belong primarily to the G protein-coupled receptor (GPCR) family and are located on both presynaptic and postsynaptic cells. Given such enduring interest in monoaminergic transmission among experimental and clinical pharmacologists, it is not surprising that the discovery of a new class of GPCRs that could be activated by a less well characterized group of endogenous amines derived from the metabolism of amino acids, termed trace amines (TAs), led to great excitement in the field (Borowsky et al., 2001; Bunzow et al., 2001).These trace amines, some of them discovered more than 100 years ago, include tyramine, tryptamine, synephrine, octopamine, and β-phenylethylamine (β-PEA) (Boulton, 1980; Sandler et al., 1980; Premont et al., 2001; Branchek and Blackburn, 2003; Berry, 2004; Miller et al., 2005; Grandy, 2007). In invertebrates, which essentially lack the norepinephrine system, octopamine and...
tyramine are recognized as major neurotransmitters/neuro-modulators and are involved in many vital functions (i.e., movement, feeding, and stress reactions). However, the role of TAs in mammalian physiology remains less well defined. In general, in vertebrates, TAs are present at lower levels in many tissues, including brain. (Boulton, 1980; Sandler et al., 1980; Branchek and Blackburn, 2003; Berry, 2004). Intriguingly, the rate of synthesis of trace amines was found to be comparable with that of classic monoamines, and low levels of TAs in brain tissue are probably determined by the extremely fast rate of metabolism and/or inability of trace amines to accumulate in substantial concentrations in synaptic vesicles (Grandy, 2007). Trace amines are structurally closely related to classic monoamines, as well as to some psychotropic molecules, such as amphetamine and related compounds. In fact, the best known trace amine, β-PEA, differs from amphetamine by one methyl group in the α-position and has been considered an "endogenous amphetamine" (Janssen et al., 1999). Although the functional role of trace amines in mammals remains largely enigmatic, it has been noted that trace amine levels can be altered in various human disorders, including schizophrenia, Parkinson's disease, attention deficit hyperactivity disorder (ADHD), Tourette syndrome, and phenylketonuria (Boulton, 1980; Sandler et al., 1980). It was generally held that trace amines affect the monoamine system indirectly via interaction with plasma membrane transporters such as plasma membrane dopamine transporter (DAT) and vesicular storage (Premont et al., 2001; Branchek and Blackburn, 2003; Berry, 2004; Sotnikova et al., 2004). Indeed, there is substantial evidence that TAs can function as "false neurotransmitters" by displacing classic biogenic amines from their storage pools in the extracellular space via amphetamine-like mechanism. Thus, it has been suspected that at physiological levels, these substances generally have only minor effect on neuronal excitability in the absence of classic monoamines but alter neuronal responses to these amine transmitters (Premont et al., 2001; Branchek and Blackburn, 2003; Berry, 2004). However, the discovery of a specific family of GPCRs, some members of which are able to be activated by TAs (Borowsky et al., 2001; Bunzow et al., 2001), has provided solid support for a direct mechanism by which TAs can modulate neuronal activity.

### Trace Amine-Associated Receptors

Although binding sites for tryptamine, tyramine, and β-PEA had been previously reported in rat brain (Boulton, 1980; Sandler et al., 1980; Berry, 2004), the identification of a family of GPCRs that could be activated by these TAs has provided direct evidence for an independent role of these amines in neurotransmission (Borowsky et al., 2001; Bunzow et al., 2001). Only two members of this receptor family, trace amine receptor 1 (TA1, now TAAR1) and trace amine receptor 2 (TA2, now TAAR4), have so far been reported to be sensitive to TAs themselves (Borowsky et al., 2001; Bunzow et al., 2001; Lindemann and Hoener, 2005), whereas other receptors were not shown to be responsive to trace amines (Lindemann and Hoener, 2005), and many TAARs are sensitive to certain volatile amines (Liberles and Buck, 2006). Based on the fact that some members of this receptor family may not respond to TAs, and partly based on the order of chromosomal localization of their genes, a novel nomenclature for these receptors was proposed that designated this receptor family as trace amine-associated receptors (TAARs) (Lindemann and Hoener, 2005). All members of this family, which also includes several previously identified "orphan GPCRs," have a unique carboxyl-terminal peptide "fingerprint" sequence (Lindemann and Hoener, 2005; Lindemann et al., 2005). TAAR genes clustered in a narrow region of approximately 100 to 200 kilobases on the same chromosome. This is reminiscent of the chromosomal localization of members of the olfactory receptor family (Liberles and Buck, 2006). Intriguingly, human TAAR genes map to chromosome locus 6q23.1, close to a major susceptibility locus for schizophrenia and bipolar disorder (Lindemann and Hoener, 2005; Lindemann et al., 2005). Phylogenetic analysis has revealed that the TAAR family is likely to have originated from a common ancestor gene that has closest similarity to the human 5-HT4 serotonin receptor gene via possible gene duplication events (Lindemann et al., 2005). There are remarkable differences in the number of genes and the proportion of intact genes relative to pseudogenes between species. Thus, although there are 19 TAAR genes with 2 pseudogenes in the rat genome, 16 genes (1 pseudogene) were found in mouse, with only 9 TAAR genes identified in human and chimpanzee genomes with 3 and 6 pseudogenes, respectively (Lindemann and Hoener, 2005; Lindemann et al., 2005). Liberles and Buck (2006) showed that essentially all TAARs, with the exception of TAAR1, are localized in the olfactory epithelium with little to no expression of any TAAR in the brain or other tissue of the body. This is quite surprising given that multiple labs found TAAR1 in various brain areas and in the periphery (Borowsky et al., 2001; Bunzow et al., 2001; Grandy, 2007; Xie et al., 2007; Lindemann et al., 2008), whereas other TAARs, such as TAAR5, TAAR6, and TAAR9, have been detected in human CNS and peripheral organs, with TAAR5 actually being found in human basal ganglia (Zeng et al., 1998; Borowsky et al., 2001). Given the fact that TA2/TAR4 is a pseudogene in the human genome (Lindemann and Hoener, 2005) and relatively little information is currently available on the biochemistry and function of other TAARs, we will focus in this assay on the TAAR1 receptor that is relatively conserved and found in all studied species.

### Biochemistry and Pharmacology of TAAR1

TAAR1 (formerly TA1) is the best characterized member of TAAR family (Grandy, 2007). TAAR1 couples to Gs, and when assessed by using cAMP accumulation, is activated by trace amines such as β-PEA and tyramine, metabolites of catecholamines and iodothyronamines, as well as several compounds known to affect monoaminergic transmission (Borowsky et al., 2001; Bunzow et al., 2001). Intriguingly, d- and l-amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), and other closely related compounds are also able to activate TAAR1 receptors in vitro as evidenced by cAMP stimulation in human embryonic kidney cells. In the mouse CNS, mRNA for TAAR1 is distributed throughout the limbic system and in regions containing catecholaminergic cell bodies and their projections—such as the locus ceruleus, substantia nigra, ventral tegmental area, dorsal raphe, striatum, and basal ganglia (Borowsky et al., 2001). In the rat, TAAR1 mRNA was found throughout the...
brain, with the highest expression in the olfactory bulb, nucleus accumbens/olfactory tubercle, cortical regions, substantia nigra, ventral tegmental area, cerebellum, and pons/medulla. In the periphery, TAAR1 mRNA was also found in the liver, kidney, gastrointestinal tract, spleen, pancreas, and heart (Bunzow et al., 2001). Significant expression of TAAR1 mRNA and protein in the primary dopaminergic areas, such as substantia nigra and striatum, was observed also in rhesus monkey brain (Xie et al., 2007). It is noteworthy that the amygdala region in rat, mouse, and human CNS contains the highest levels of TAAR1 mRNA (Borowsky et al., 2001). The TAAR1 expression pattern in the mouse brain has been analyzed using the LacZ reporter inserted in the Taar1 gene in frame with the endogenous start codon (Lindemann et al., 2008). This sensitive approach revealed specific labeling of predominantly dopaminergic and serotonergic brain areas, such as hypothalamus and preoptic area, ventral tegmental area, amygdala, dorsal raphe nucleus, nucleus of the solitary tract, and in the parahippocampal region (rhinal cortices) and subiculum (Lindemann et al., 2008). Taken together, these data convincingly demonstrate that TAAR1 is widely expressed in the primary monoaminergic areas of the brain and well positioned to modulate locomotor, emotional, and motivated behaviors that are traditionally associated with monoaminergic activity.

The study of TAAR1 pharmacology in heterologous cellular systems in vitro has been challenging, however, primarily because of the phenomenon that TAAR1 remains largely intracellular when heterologously expressed in cell lines (Bunzow et al., 2001; Miller et al., 2005; Wolinsky et al., 2001; Grandy, 2007; Reese et al., 2007; Wainscott et al., 2007; Barak et al., 2008). In general, with such relatively low level of expression in heterologous cellular systems, TAAR1 displays a reduced signaling capability that can be restored by replacing parts of the receptor sequence or the stimulatory G protein with the corresponding rat counterparts, coexpression with rat Gαq, promiscuous Gq, or Gα16 (Bunzow et al., 2001; Lindemann and Hoener, 2005; Reese et al., 2007; Wainscott et al., 2007). The use of a highly sensitive cAMP response element-luciferase reporter-based assay may also circumvent this problem (Miller et al., 2005; Xie and Miller, 2007; Xie et al., 2007). This is particularly relevant for human TAAR1, which has proven to be notoriously difficult to express in heterologous cellular system; additional modifications to the receptor are necessary to reliably express it in cells. Even with such modifications (Bunzow et al., 2001; Miller et al., 2005; Wolinsky et al., 2006; Reese et al., 2007; Wainscott et al., 2007), relatively little membrane expression but predominant intracellular expression pattern was achieved. This is particularly important for pharmacological experiments, because receptor density and G protein abundance can both affect apparent agonist potency and efficacy in vitro. 

In vitro cellular assays (Kenakin and Morgan, 1989). Although recent reports indicate that permanent cell lines expressing hTAAR1 could be achieved (Navarro et al., 2006; Wainscott et al., 2007; Levin et al., 2008), the problem with reliable plasma membrane expression of TAAR1 and other TAAR receptors still remains a major concern in cellular pharmacological studies (Grandy, 2007; Barak et al., 2008).

Despite these difficulties, several groups have confirmed the original observations demonstrating potent and full agonist activity of the trace amine β-PEA and tyramine on TAAR1 with EC50 in the range of 40 to 900 nM for β-PEA and 70 to 1100 nM for tyramine (Borowsky et al., 2001; Bunzow et al., 2001; Lindemann and Hoener, 2005; Miller et al., 2005; Navarro et al., 2006; Wolinsky et al., 2006; Grandy, 2007; Reese et al., 2007; Wainscott et al., 2007; Barak et al., 2008; Levin et al., 2008). A relatively weaker activity of octopamine and tryptamine at the rat and human TAAR1 has been described (Borowsky et al., 2001; Bunzow et al., 2001; Lindemann and Hoener, 2005; Miller et al., 2005; Navarro et al., 2006; Wainscott et al., 2007; Barak et al., 2008). It should be noted, however, that substantial differences in potencies of putative TAAR1 ligands between TAAR1 receptors of different species have been observed. In general, it was found that that β-PEA is a more potent agonist than tyramine at the human and mouse TAAR1, whereas at the rat TAAR1, tyramine is more potent than β-PEA (Grandy, 2007). Profiling of structure activity for a number PEA-related compounds has yielded much needed information with regard to specific molecular modifications of the aromatic moiety and ethylene chain of β-PEA that could enhance or reduce activity of compounds at the hTAAR1 (Wainscott et al., 2007; Levin et al., 2008).

Another tantalizing observation made by Bunzow et al. (2001) has indicated that the meta-O-methyl metabolites of catecholamines such as 3-methoxytyramine (3-MT), 4-methoxytyramine (4-MT), normetanephrine, and metanephrine can exert potent agonistic activity at the rat TAAR1. Other groups have confirmed the activity of 3MT (Wainscott et al., 2007; Barak et al., 2008) and 4-MT (Barak et al., 2008) at the human TAAR1. 3-MT is a well known extracellular metabolite of dopamine generated as a result of catechol-O-methyl transferase (COMT) activity. It is commonly considered a biologically inactive compound, and its concentration in the tissue was generally interpreted as a marker of extracellular dopamine (Kehr, 1976). Identification of activity of these metabolites at TAAR1 strongly suggests that 3-MT and other products of COMT could play important neuromodulatory roles in certain brain regions in which catecholamines are abundant. Characterization of in vivo activities of these metabolites at the endogenous TAAR1 in animals and understanding of their signaling and behavioral profiles could result in an important breakthrough in understanding catecholamine biology. In addition, these data indicate that COMT may not simply be a metabolizing enzyme but can also serve as rate-limiting step for these potential neuromodulators (Bunzow et al., 2001). It would be of great interest to explore whether the activities of 3-MT and other metabolites have some pathophysiological roles in conditions when their concentrations might be abnormally increased, such as in dyskinetic Parkinson’s disease patients after long-term L-DOPA treatment (Rajput et al., 2004). Finally, identification of neuromodulator/neurotransmitter function of 3-MT would further support the increasingly appreciated concept that not only major products of synthetic pathways but also their degradation products can serve as signals to activate specific receptive systems to provide an organism with plethora of signals to fine-tune modulation of its functions.

Another group of endogenous compounds that can activate TAAR1 are represented by 3-iodothyronamine (T1AM) and its deiodinated relative thyronamine, molecules that are believed to be derived from thyroid hormone (Scanlan et al., 2004; Hart et al., 2006; Tan et al., 2007). It has been shown that T1AM and its enantiomer thyronamine are potent full...
Amphetamines share a similar chemical structure with the endogenous trace amine β-phenylethylamine, a similarity actually reflected in the full chemical name of amphetamine—Alpha-Methyl-Pβ-Phenylethylamine. The fact that β-PEA has the best established activity at TAAR1 to date (Borowsky et al., 2001; Bunzow et al., 2001; Lindemann and Hoener, 2005) has prompted investigation of whether amphetamines can have direct activity at TAAR1 (Bunzow et al., 2001). These initial studies have indicated that several amphetamine derivatives, including d-amphetamine, l-amphetamine, d-methamphetamine, and (+)-MDMA, can induce increase in cAMP in human embryonic kidney cells stably expressing rat TAAR1 receptors. Several other groups have confirmed these observations with mouse, rat, rhesus monkey, and human TAAR1 receptors expressed in various heterologous cellular systems (Miller et al., 2005; Wolinsky et al., 2006; Reese et al., 2007; Wainscott et al., 2007; Xie and Miller, 2007; Xie et al., 2007; Barak et al., 2008; Lewin et al., 2008). All of these studies have convincingly demonstrated that many amphetamine derivatives can activate TAAR1 receptor in vitro with potencies shown in Table 1. It is important to note that the concentration range of d-amphetamine (0.136–1.12 μM) and MDMA (0.370 μM) active at human TAAR1 are within the plasma concentration levels achievable by administration of amphetamine to humans [approximately 300 nM (Asghar et al., 2003)] or MDMA to primates [approximately 6–12 μM (Bowyer et al., 2003) for review, see Grandy, 2007]. In chronic amphetamine or methamphetamine abusers, plasma concentrations of both drugs can reach into the high micromolar range (Peters et al., 2003). It should be noted also that interaction of various amphetamines with the DAT was found to be in range of 0.025 to 2.650 μM (Rothman et al., 2001). Thus, it is highly likely that the activation of TAAR1 by amphetamine derivatives may have significant physiological consequences in vivo, and TAAR1 may play a role in neuronal processes that are important for amphetamine-related disorders or therapeutic responses to amphetamine and related compounds.

Among other compounds that have been reported to activate TAAR1 are most noticeable ergolines, including lysergic acid diethylamide (LSD), the DAT blocker nomifensine, the DA agonists apomorphine and bromocriptine, and several others (Borowsky et al., 2001). However, further independent confirmations are necessary to support these intriguing observations and evaluate whether some pharmacological effects of these compounds could be mediated by TAAR1. Strikingly, with such an extensive number of TAAR1 agonists identified by several independent groups, no potent TAAR1 antagonists have yet been reported. It should be mentioned, however, that recent attempts to apply structure-based drug design using the rotamer toggle switch model of aminergic GPCR activation has resulted in identification of several weak TAAR1 agonists among T1AM derivatives (at a potency of 3–5 μM) that could serve as lead compounds for development of potent and selective TAAR1 antagonists (Tan et al., 2008). Identification of such TAAR1 antagonists would be critical for future progress in the field, and these developments are eagerly awaited.

In our laboratory (Barak et al., 2008), in an attempt to identify agonists and antagonists of TAAR1 we initially used a high content high throughput cell assay employing β-arrestin2 (βarr2) molecules labeled with green fluorescent pro-

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<tr>
<th>Studies and Receptors Studied</th>
<th>d-Amphetamine</th>
<th>l-Amphetamine</th>
<th>d-Methamphetamine</th>
<th>MDMA</th>
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<td>μM</td>
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<td>Bunzow et al. (2001); Reese et al. (2007)</td>
<td>0.21</td>
<td>4.96</td>
<td>0.92</td>
<td>1.700(±-MDMA)</td>
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<td>mTAAR1</td>
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<td>0.89</td>
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<td>3.09</td>
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<td>Miller et al. (2005); Xie and Miller (2007); Xie et al. (2007)</td>
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<td>1.700</td>
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<td>0.245</td>
<td>1.31</td>
<td>0.370(+/-MDMA)</td>
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mTAAR1, mouse TAAR1; rTAAR1, rat TAAR1; rhTAAR1, rhesus monkey TAAR1; h-rTAAR1, chimera human-rat TAAR1; hTAAR1, human TAAR1
tein (βar-GFP) (Barak et al., 1997; Kim et al., 2001; Barak et al., 2003, 2008). βar-GFP binds to agonist-activated GPCRs and competitively blocks G protein-dependent receptor signaling (Lefkowitz et al., 1993; Barak et al., 1997; Barak et al., 2003). This desensitization process, in which βar-GFP redistributes in response to agonists and is prevented from redistributing by coexpression of antagonists, can be readily measured using automated high-content imaging tools. Inherent to the success of the βar-GFP assay is an adequate cellular expression of the receptors and the ability of these receptors to desensitize. To express the human TAAR1 in sufficient quantity to enable this screening, we first engineered hTAAR1 receptor that also included the first nine amino acids of the human β2-adrenergic receptor (β2-AR). This insert contains a consensus glycosylation site at position 6, known to be critical to enhance β2-AR expression at the plasma membrane (Rands et al., 1990). With this modification, we achieved significant plasma membrane localization of hTAAR1 at levels sufficient for characterizing receptor biology and developing reliable in vitro cellular assays. Nevertheless, although we achieved clear plasma membrane TAAR1 expression, these levels are still 5- to 10-fold lower than those for the human β2-AR (Barak et al., 2008). Thus, we observed that although hTAAR1 was able to recruit β-arrestin 2, similar to other GPCRs, this modest translocation resembled that of the dopamine D3 receptor, which is known to desensitize relatively poorly in a βar-dependent manner (Kim et al., 2005). This essentially prevented us from using the βar2 translocation assay, and we developed a novel protocol for high-throughput screening of small-molecule libraries for potential hTAAR1 ligands in vitro by using newly developed BRET cAMP assay (Barak et al., 2008). cAMP measurements were performed by using a bioluminescence method that is suitable for GPCR activation analysis (Gulés et al., 2005). In this assay, the amount of light emitted by the fluorescent protein decreases with cAMP binding to the cAMP sensor protein known as exchange protein activated by cAMP (EPAC) (Jiang et al., 2007; Barak et al., 2008; Masri et al., 2008). Remarkably, the TAAR1 is well suited to this assay, as demonstrated by time course and dose response measurements of the bioluminescence. The time course of signaling showed that the intracellular concentration of cAMP is stable over long periods after activation of the receptor, further indicating that TAAR1 desensitizes rather poorly. This is in contrast to the β2-AR, in which the production of cAMP decreased after 5 min and the curve returned to its preactivation baseline as a result of desensitization of the receptor (Barak et al., 2008). In this assay, we initially measured the dose response of approximately 40 compounds that could be potential ligands of the TAAR1 either as agonists or as antagonists. Although we did not detect any antagonists among these compounds, based on their ability to counteract β-PEA-induced BRET signal, we found several compounds (including β-PEA, tyramine, 3-MT, 4-MT, and amphetamines) that induced potent agonist activity, supporting previous reports on the activity of these compounds at TAAR1 receptors (Barak et al., 2008). Results on hTAAR1 activity of d-amphetamine, l-amphetamine, d-methamphetamine, and (+)-MDMA are shown in Table 1. Approximately 1000 additional compounds from small molecule libraries (LoPack, Sigma libraries) were screened using this assay, and several new compounds (agonists, but no antagonists) potentially activating TAAR1 have been identified. It is noteworthy that this unbiased screening revealed tyramine, 3-MT, and 4-MT among the newly identified compounds, thus further validating this screening approach (unpublished observations).

**TAAR1 Knockout Mice.** The first insight into the functional role of TAAR1 receptors in physiological settings was gained by using mice lacking TAAR1 [TAAR1-knockout (KO) mice]. Thus far, all of the endogenous and small-molecule ligands reported to act as agonists at TAAR1 also have actions in the CNS at sites other than the TAAR1, such as DAT and vesicular monoamine transporter 2. In the absence of selective ligands for TAAR1, mice lacking TAAR1, TAAR1-KO mice represent the only available way to evaluate the possible physiological consequences of TAAR1 dysfunction and role of TAAR1 in actions of pharmacological agents. In the initial study, it has been observed that TAAR1-KO mice, developed on mixed C57BL/6J × 129S1/Sv background, had no overt phenotype, breed successfully, and showed no major differences from normal littermates in most neurological and behavioral tests (Wolinsky et al., 2006). However, it has been found that TAAR1-KO mice have a significant deficit in prepulse inhibition test, indicating an impairment of sensorimotor gating that is known to be deficient in patients with schizophrenia and several other brain disorders (Wolinsky et al., 2006). Furthermore, it has been found that TAAR1-KO mice have enhanced sensitivity to the locomotor stimulating effects of d-amphetamine and show greater increase in extracellular monoamine levels after d-amphetamine in in vivo microdialysis studies (Wolinsky et al., 2006). Essentially the same major observations were made in C57BL/6J congenic TAAR1-deficient mice developed independently by Hoener’s group (Lindemann et al., 2008). These mutants also showed increased sensitivity to amphetamine both in locomotor activity test and displayed augmented striatal dopamine release after amphetamine. Furthermore, while under baseline conditions, no significant changes in locomotor activity and extracellular dopamine levels were found, and electrophysiological recordings in the ventral tegmental area revealed an elevated spontaneous firing rate of dopaminergic neurons in TAAR1-KO mice (Lindemann et al., 2008).

We have established a colony of TAAR1-KO mice (generous gift of Lundbeck Research USA, Paramus, NJ) and have performed some additional experiments. In our settings, we noted a minor hyperactivity of TAAR1-deficient mice in a novel environment and enhanced locomotor responses to amphetamine but also to β-PEA and MDMA in both knockout and heterozygous TAAR1 mutant mice. In addition, intriguingly, we observed that haloperidol-induced catalepsy is markedly reduced in TAAR1-deficient mice (T. D. Sotnikova and R. R. Gainetdinov, unpublished observations).

Many TAAR1 ligands, including β-PEA and amphetamines, demonstrate potent interaction with the DAT, thereby significantly complicating elucidation of TAAR1-mediated actions. Mice lacking the DAT provide a unique opportunity to investigate DAT-independent actions of compounds (Giros et al., 1996; Gainetdinov and Caron, 2003). Furthermore, DAT-deficient mice provide a model to investigate the inhibitory actions of amphetamines on hyperactivity, the feature of amphetamines believed to be important for their therapeutic action in ADHD (Gainetdinov et al., 1999;
Gainedtдинov and Caron, 2003). It should be noted also that the best-established agonist of TAAR1, \( \beta \)-PEA, shared the ability of amphetamine to induce inhibition of dopamine-dependent hyperactivity of DAT-KO mice (Gainetdinov et al., 1999; Sotnikova et al., 2004). Therefore, we attempted to generate mice lacking both the TAAR1 and DAT and directly assess the effects of amphetamine in these double mutants. Double DAT/TAAR1-null mutant mice are viable and display significantly enhanced levels of spontaneous locomotor activity compared with parental DAT-KO mice. Strikingly, both \( \beta \)-amphetamine and \( \beta \)-PEA, which produce the inhibitory action in DAT-KO mice (Gainetdinov et al., 1999; Sotnikova et al., 2004), were substantially less potent in this regard in double mutants (T. D. Sotnikova and R. R. Gainedtдинov, unpublished observations). These and other pilot experiments (Sotnikova et al., 2008) suggest that TAAR1 may play an inhibitory role with regard to DA-dependent locomotor hyperactivity, and thus TAAR1 deficiency or antagonism could enhance DA-dependent behaviors and functions while TAAR1 agonists could inhibit it.

With regard to mechanism of TAAR1 involvement in the action of amphetamines, and regulation of monoaminergic transmission in general, three possibilities are currently under investigation. First, TAAR1 may directly interact with the DAT, thereby affecting DA neurotransmission via modulation of DAT function. Recent studies have demonstrated coexpression of TAAR1 with DAT in a subset of dopamine neurons in both mouse and rhesus monkey substantia nigra, and monoamine transporter-modulated activation of TAAR1 (Xie and Miller, 2007; Xie et al., 2007). Such interaction potentially may explain the enhanced outflow of DA after amphetamine in TAAR1-KO mice (Wolinsky et al., 2006). A second possibility suggests that TAAR1 localized at cell bodies of monoaminergic neurons may directly modulate activity of these neurons in response to various compounds active at TAAR1 (Mercuri and Bernardi, 2005; Lindemann et al., 2008). Finally, it is possible that TAAR1 directly interacts with dopamine receptors as a heterodimer or at the level of signaling intermediates, thereby providing important modulatory influence over dopaminergic neurotransmission. In fact, it has been observed that TAAR1 activation by high concentrations of dopamine can be blocked by concurrent D2 activation and that the D2 effects are reversible by the D2 antagonist raclopride, suggesting that TAAR1 activation can be regulated by the D2 dopamine receptor when both receptors are coexpressed in the same cell (Xie et al., 2007). Furthermore, a higher proportion of D2 receptors in a high-affinity state was observed in TAAR1-KO mice (Wolinsky et al., 2006). Disrupted haloperidol catalepsy in TAAR1-KO mice also supports this hypothesis. Further detailed investigations will be necessary to understand the neurochemical mechanisms involved in the modulation of dopaminergic function by TAAR1.

**Future Directions**

The major challenge for future research is to understand the role of TAAR1 in the physiological functions and actions of biologically active compounds in vivo. These investigations could provide critical information with regard to therapeutic potential of future TAAR1 agonists and antagonists. In vitro cellular work has established that some trace amines, amphetamines, and monoamine metabolites are potent and efficacious agonists of TAAR1 with specific concentration-dependent and species-dependent stereospecific pharmacological profiles. For example, given the fact that the EC\textsubscript{90} values for such actions of amphetamines are comparable with the concentrations found in some human addicts, it is highly likely that TAAR1 mediates some specific effects of amphetamines in humans (Grandy, 2007). This suggests also that genetic variants in human TAAR1 may be an important factor predisposing some people to amphetamine abuse or other amphetamine-related disorders. Furthermore, if TAAR1 could be proven as a mediator of some of amphetamine’s actions in vivo, the development of novel TAAR1-selective agonists and antagonists could provide a new approach for the treatment of amphetamine-related conditions such as addiction and/or disorders in which amphetamine is used therapeutically. In particular, because amphetamine has remained the most effective pharmacological treatment in ADHD for many years, a potential role of TAAR1 in the mechanism of the “paradoxical” effectiveness of amphetamine in this disorder should be explored.

It would be of interest as well to investigate whether TAAR1 activation could contribute to the development of psychotic reactions observed after high doses of amphetamines. In general, because it seems that TAAR1 is involved in modulation of monoaminergic transmission and can exert a tonic inhibitory action in locomotor activity tests, future TAAR1-selective drugs could find usefulness in disorders traditionally associated with dopaminergic dysfunction, such as schizophrenia and Parkinson’s disease. Observation of a major endophenotype of schizophrenia, deficit in sensorimotor gating, in TAAR1-deficient mice and multiple lines of evidence of intimate interaction between TAAR1 and dopaminergic system strongly encourage studies aimed at evaluation of the role of TAAR1 as a potential new target for treatment of this disorder.

In addition, given that TAAR1 seems to play generally an inhibitory role with regard to locomotor activity and the fact that several \( O \)-methylated metabolites of catecholamines, including the extracellular DA metabolite 3-MT, can activate TAAR1, understanding the potential role of TAAR1 in Parkinson’s disease would be particularly interesting. It might be expected that the blockade of TAAR1 by antagonists could provide a novel approach to enhance the antiparkinsonian action of \( L \)-DOPA. Clearly, further experiments will be necessary to explore these possibilities in detail, and, investigation of the role of TAAR1 in the actions of short- and longer-term \( L \)-DOPA administration would be particularly informative. Given the widespread expression of TAAR1, not only in the brain but also in the periphery, with documented presence in the heart, kidney, liver, gastrointestinal tract, spleen, and pancreas (Grandy et al., 2007), it would not be unreasonable to propose that future TAAR1-specific compounds could eventually find therapeutic potentials in disorders involving these organs as well.

In general, because an imbalance in the function of TAs might have important implications in the pathology of many disorders, finding selective small molecule agonists and antagonists for TAARs could become a new approach in search for new pharmacologically active compounds. Although TAAR1 has been the subject of intensive investigations over the last several years, studies aimed at identification of spe-