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REVIEW

Trace amine-associated receptors and their ligands

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Classical biogenic amines (adrenaline, noradrenaline, dopamine, serotonin and histamine) interact with specific families of G protein-coupled receptors (GPCRs). The term 'trace amines' is used when referring to *p*-tyramine, β-phenylethylamine, tryptamine and octopamine, compounds that are present in mammalian tissues at very low (nanomolar) concentrations. The pharmacological effects of trace amines are usually attributed to their interference with the aminergic pathways, but in 2001 a new gene was identified, that codes for a GPCR responding to *p*-tyramine and β-phenylethylamine but not to classical biogenic amines. Several closely related genes were subsequently identified and designated as the trace amine-associated receptors (TAARs). Pharmacological investigations *in vitro* show that many TAAR subtypes may not respond to *p*-tyramine, β-phenylethylamine, tryptamine or octopamine, suggesting the existence of additional endogenous ligands. A novel endogenous thyroid hormone derivative, 3-iodothyronamine, has been found to interact with TAAR1 and possibly other TAAR subtypes. *In vivo*, micromolar concentrations of 3-iodothyronamine determine functional effects which are opposite to those produced on a longer time scale by thyroid hormones, including reduction in body temperature and decrease in cardiac contractility. Expression of all TAAR subtypes except TAAR1 has been reported in mouse olfactory epithelium, and several volatile amines were shown to interact with specific TAAR subtypes. In addition, there is evidence that TAAR1 is targeted by amphetamines and other psychotropic agents, while genetic linkage studies show a significant association between the TAAR gene family locus and susceptibility to schizophrenia or bipolar affective disorder.

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Abbreviations: AADC, aromatic L-amino acid decarboxylase; DOI, 2-amino,1-[2,5-dimethoxy-4-iodophenyl]-propane; GPCR, G protein-coupled receptor; MAO, mono amino oxidase; MDMA, 3,4-methylenedioxymetamphetamine; MTPT, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; T_0AM, thyronamine; T_1AM, 3-iodothyronamine; T_3, 3,5,3′-triiodothyronine; T_4, thyroxine; TAAR, trace amine-associated receptor

Introduction

In the last 5 years, important discoveries have been made in the field of aminergic signaling. Beginning in 2001, a new family of G protein-coupled receptors (GPCRs) was reported (Borowsky *et al.*, 2001; Bunzow *et al.*, 2001). Members of this large family of GPCRs are now referred to as trace amine-associated receptors or TAARs based on the pharmacological profile of its prototypical member, TAAR1 (Lindemann *et al.*, 2005). In 2004, some amines derived from thyroid hormone decarboxylation and deiodination, known as thyronamines, were shown to be endogenous compounds that stimulate cAMP production via activation of heterologously expressed mouse and rat TAAR1 (Scanlan *et al.*, 2004). Recently, it has been suggested that TAARs may represent a class of chemosensory receptors in the olfactory epithelium (Liberles and Buck, 2006). The discovery of a novel family of receptors and a novel complement of endogenously produced ligands throws open completely new perspectives, from biochemistry to cell biology, impacting physiology, pharmacology and pathophysiology of the thyroid gland, brain, olfactory sensory neurons and likely other organ systems, as well. The present review summarizes the key new developments in this field. For additional background information, the reader is referred to recent reviews on aminergic receptors (Shi and Javitch, 2002; Hill, 2006), trace amines (Premont *et al.*, 2001; Davenport, 2003; Berry, 2004), and thyroid hormone metabolism (Wu *et al.*, 2005). Reviews focussed on trace amine receptors have also appeared (Branchek and Blackburn, 2003; Lindemann and Hoener, 2005; Lindemann *et al.*, 2005; Lewin, 2006).

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Biological amines and trace amines

The term 'biogenic amines' was introduced to designate a collection of amines that exert important biological effects as chemical messengers, that is, as hormones, local hormones, neuromodulators or neurotransmitters (Figure 1). They are all produced from aromatic amino acids and their synthetic pathways include a decarboxylation step that is catalyzed by one of several aromatic amino acid decarboxylases. The first biogenic amines to be discovered were the catecholamines, that is adrenaline (epinephrine), noradrenaline (norepinephrine) and dopamine, which derive from tyrosine. Other well-known biogenic amines are histamine and serotonin (5-hydroxytryptamine), which are derived from histidine and tryptophan, respectively.

The catecholamines noradrenaline and adrenaline were soon recognized to interact with cell surface membrane proteins known collectively as GPCRs (Dixon *et al.*, 1986; Lefkowitz, 2004). Other metabotropic, aminergic GPCRs have subsequently been identified for dopamine, histamine and serotonin, although with the latter interacting also with an ionotropic receptor, the 5-HT3.

Other biogenic amines are present in the central nervous system at very low concentrations in the order of 0.1–10 nM, representing <1% of total biogenic amines (Berry, 2004). For these compounds, the term 'trace amines' was introduced. Although somewhat loosely defined, the molecules generally considered to be trace amines include *para*-tyramine, *meta*-tyramine, tryptamine, *β*-phenylethylamine, *para*-octopamine and *meta*-octopamine (Berry, 2004) (Figure 2).

Contrary to the situation in vertebrates, some trace amines are the chief amines found in many invertebrate species (Axelrod and Saavedra, 1977; David and Coulon, 1985; Evans and Robb, 1993; Roeder, 1999; Saraswati *et al.*, 2004); for instance, octopamine and tyramine are thought to be the chief insect neurotransmitters, and octopamine is regarded as the sympathetic nervous system counterpart to noradrenaline in invertebrates. Trace amines are also produced in bacteria, fungi, plant cells, and can be found in some foods, most notably chocolate, cheese and red wine (Branchek and Blackburn, 2003).

The functional effects of trace amines have been traditionally referred to as 'sympathomimetic' (Brunton *et al.*, 2005). Some of these sympathomimetic symptoms appear when sensitive subjects treated with inhibitors of monoamine oxidases (the enzymes primarily responsible for trace amine degradation: see below) consume foods that contain high concentrations of trace amines. In the central nervous system, trace amines can produce amphetamine-like responses, for example increased alertness, euphoria, irritability, decreased appetite, insomnia and tremor. Cardiovascular responses include tachycardia and vasomotor effects, which may lead to either hypertension or hypotension, depending on the dosage, experimental/clinical conditions and on the organism being studied. Nausea, emesis, decreased or increased bronchial resistance, sweating, hyperthermia and headache have also been reported. In the kidney, tyramine can increase chloride permeability (Blumenthal, 2003) and urinary flow (Rahman *et al.*, 1995), even when the blood flow is unchanged. In contrast, octopamine decreases sodium and water excretion (Levy, 1988). Endocrine effects have also been reported: *p*-tyramine, *β*-phenylethylamine and octopamine can each inhibit prolactin secretion in anterior pituitary cells grown *in vitro* and *in vivo* (Becu-Villalobos *et al.*, 1992); *β*-phenylethylamine increases ACTH and glucocorticoid production in rats (Kosa *et al.*, 2000); octopamine produces an insulin-like effect on glucose uptake in adipocytes, skeletal muscle and myocardium (Visentin *et al.* 2001; Morin *et al.*, 2002).

The above-described effects are typically produced by micromolar concentrations of trace amines, and their physiological relevance has therefore been questioned. At submicromolar concentrations, trace amines have been reported to produce neuromodulatory actions; that is, they either increase or decrease a cell's response to different neurotransmitters. As reviewed elsewhere (Berry, 2004), *β*-phenylethylamine and *p*-tyramine potentiate the

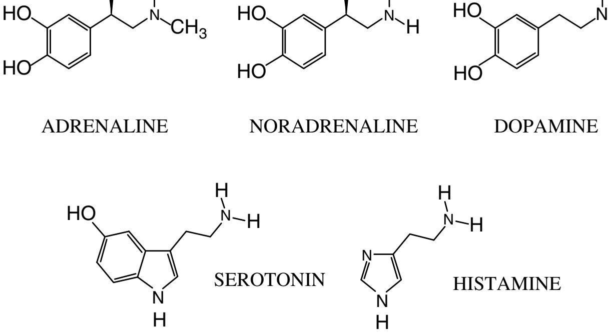

Figure 1 Structures of the classical biogenic amines.

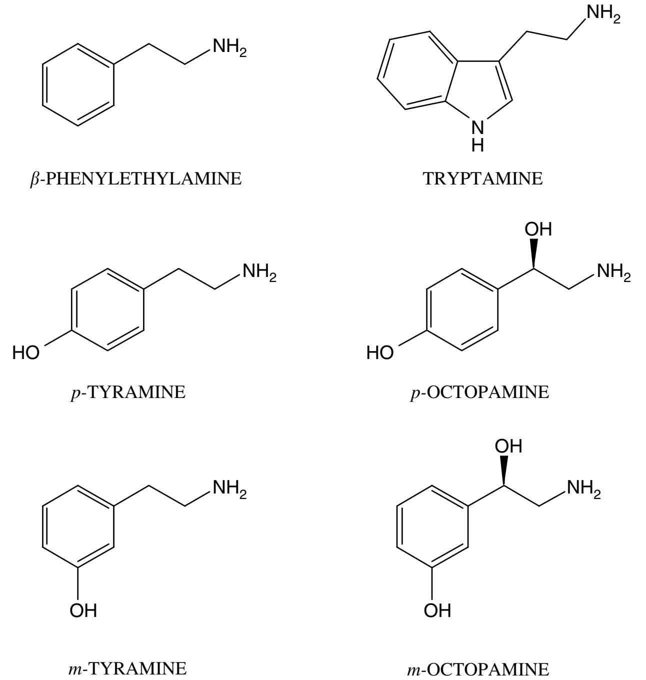

Figure 2 Structures of the compounds usually referred to as 'trace amines'.

responses elicited by noradrenaline and dopamine, whereas octopamine selectively increases neuronal responses to noradrenaline, while tryptamine potentiates the inhibitory response of cells to serotonin. In the neocortex of reserpinized rats atropine-resistant, low-voltage fast activity was restored by β-phenylethylamine (Vanderwolf *et al*., 1980). Inhibitory actions of trace amines have also been observed: in rat midbrain slices, β-phenylethylamine and *p*-tyramine depressed the inhibitory postsynaptic response mediated by GABA (B) receptors, possibly by interfering with the coupling of the receptor to inward rectifying potassium channels (Federici *et al*., 2005). In addition, β-phenylethylamine, *p*-tyramine and octopamine inhibited the electrically evoked release of acetylcholine in rat striatum, an effect that required the integrity of the striato-nigral dopaminergic system (Baud *et al*., 1985). In contrast, intraperitoneal administration of β-phenylethylamine increased striatal acetylcholine release, possibly through a glutamatergic pathway (Ishida *et al*., 2005). Whether these neuromodulatory actions have any physiological role remains to be determined.

Over the years, a pathophysiological role for trace amines in the genesis of several neurological or mental disorders has been advocated. Deficiency in β-phenylethylamine has been correlated with depression, and decreased β-phenylethylamine catabolism has been associated with schizophrenia. Individuals suffering from Parkinson's disease or those diagnosed with attention-deficit hyperactivity disorder may benefit from β-phenylethylamine administration (Schildkraut, 1976; Usdin and Sandler, 1976; Reynolds, 1979; Boulton, 1980; Davis and Boulton, 1994). Interestingly, it has been suggested that *p*-tyramine is essential for sensitization to cocaine in *Drosophila* (McClung and Hirsh, 1999). Traditionally, it has been taught that *p*-tyramine plays a role in the development of migraine headache; however, the veracity of this connection remains controversial. Foods rich in tyramine, such as chocolate, have been thought for a long time to trigger migraine attacks, but direct clinical studies have failed to demonstrate a firm link between migraines and tyramine exposure to date (Branchek and Blackburn, 2003; D'Andrea *et al*., 2004). A pathophysiological role for trace amines in hepatic encephalopathy has also been proposed (Mousseau and Butterworth, 1995b).

At the molecular level, the pharmacological effects of the trace amines have traditionally been attributed to their ability to induce noradrenaline release from sympathetic nerve endings, and, in general, to compete for catecholamine or serotonin-binding sites on their cognate receptors, transporters or storage sites (Raiteri *et al*., 1977; Jones, 1983; Locock *et al*., 1984; McCormack *et al*., 1986; Parker and Cubeddu, 1988; Dyck, 1989; Paterson *et al*., 1990). The biogenic monoamine transporters in particular have received the most attention in recent years, since concrete evidence for the existence of cell surface receptors specific for trace amines has been lacking in spite of the fact that the existence of high-affinity trace amine-binding sites was documented in the mammalian brain and other tissues (particularly the liver) over 20 years ago (Hauger *et al*., 1982; Cascio and Kellar, 1983; Bruning and Rommelspacher, 1984; Vaccari, 1986; Nguyen and Juorio, 1989; Mousseau and Butterworth, 1995a). However, this simple picture of how trace amines manifest their physiological and behavioral effects must be substantially modified with the recent discovery of a new family of GPCRs that are reported to be specific for trace amines.

Trace amine-associated receptors

The discovery of the first trace amine-associated receptor (TAAR1) was made independently by two groups of investigators, Borowsky *et al*. (2001) and Bunzow *et al*. (2001), each using a similar approach. Complex mixtures of oligonucleotides whose sequences were chosen based on GPCRs for serotonin (Borowsky *et al*., 2001) or dopamine (Bunzow *et al*., 2001) were used to amplify novel DNA sequences by PCR using rat cDNA and genomic DNA as templates. Both groups independently reported the cloning of several novel and unique sequences not present in any of the publicly accessible databases. The cloned sequences predicted a 332 amino-acid protein that had all of the hallmarks an aminergic GPCR. Expression of the putative orphan receptor in either *Xenopus* oocytes or human embryonic kidney (HEK) cells revealed that it can couple to the stimulation of cAMP production upon exposure to *p*-tyramine or β-phenylethylamine. Classical biogenic amines, including the catecholamines dopamine, noradrenaline and adrenaline, as well as serotonin and histamine are either ineffective or much less potent with EC_{50} values two orders of magnitude higher than the trace amines. Based on its pharmacological profile and functional coupling to cAMP production, Borowsky *et al*. and Bunzow *et al*. concluded that their orphan GPCR was a *bone fide* receptor for trace amines.

Bunzow *et al*.'s study (2001) focused on the cloning of both rat and human trace amine receptor sequences and on extensive pharmacological characterization of the cloned rat trace amine receptor stably expressed heterologously in HEK cells. Borowsky *et al*. (2001) also performed numerous degenerate oligonucleotide-primed PCRs using rat and human genomic DNAs as templates and identified 15 sequences with high homology to each other. Seventy-four amino-acid residues are completely conserved in all 15 genes, and 52 of these are unique to this GPCR family. The latter are scattered over the whole molecule and in particular they are present in all the seven transmembrane segments, but it is still unclear as to which of these are included into the ligand-binding pocket.

The availability of nearly complete genomic sequences for several vertebrate and invertebrate species recently enabled two groups working independently to assemble what is likely to be a complete catalog of all trace amine receptor genes including how many there are in each species, their chromosomal localization, their orientation relative to one another and the presence or absence of introns (Lindemann *et al*., 2005; Gloriam *et al*., 2005b). From the deduced amino-acid sequences of the human, mouse, rat and chimpanzee TAARs, Lindemann *et al*. extracted a polypeptide sequence whose motif is characteristic of every member of this large receptor family and 100% specific when used to query all current SwissProt entries. This motif overlaps with what is considered to be the receptor's putative seventh transmembrane domain and is defined as NSXXNPXX[YH]XXX[YF]XWF.

Collectively, 53 genes have been reported by genome-scanning efforts with nine of them in humans (including three pseudogenes); nine in chimpanzees (including six pseudogenes); 19 in rats (including two pseudogenes); and 16 in mice (including one pseudogene). As only one member of this family of GPCRs clearly responds to trace amines, at least *in vitro* (see below), Lindemann *et al*. (2005) proposed that all members of the family be referred to as 'trace amine-*associated* receptors' (TAARs). In addition, Lindemann *et al*. (2005) proposed that each receptor gene be given a unique identifier that reflects where it is physically located on the chromosome relative to TAAR1, what other TAAR sequences it is most closely related to and if it is likely to be a pseudogene or not (Table 1). The human and chimpanzee genomes include nine TAAR genes, and the labels TAAR1 to TAAR9. The rat and mouse genomes contain additional genes that on the basis of sequence homologies, appear to have been generated by duplication events within the lineage of each species. Such genes are called paralogue genes and are identified by a letter suffix, while genes with the same number, which were likely generated by speciation events, are called orthologous genes. For instance, the rat genome contains three genes that are orthologues of the human TAAR8 and are designated: TAAR8a, TAAR8b and TAAR8c; and nine genes that are orthologues of human TAAR7 and are designated: TAAR7a to TAAR7i (Lindemann *et al*., 2005).

All members of the trace amine receptor gene family produce relatively short transcripts with characteristically short coding regions (~1000 base pairs in length) derived from a single exon. The exception to this 'rule' is TAAR2, a receptor whose gene consists of two coding exons. Furthermore, in all mammalian species analyzed to date members of this gene family are linked along a stretch of a single chromosome. In humans, all of the trace amine receptor genes are located on chromosome 6 at band q23.1; in rat, the family is clustered on chromosome 1p12; and, in mouse, the family resides on chromosome 10 (Lindemann *et al*., 2005).

Other vertebrate genera in which TAARs have been identified include the fish species *Takifugu rupbripres* (fugu) and *Danio rerio* (zebrafish). In contrast to mammals, the zebra fish repertoire of 57 trace amine GPCR genes is scattered among at least six chromosomes (Gloriam *et al*., 2005b). In addition to their extensive analysis of the *Danio* genome, Gloriam *et al*. (2005a, b) also made a surprising observation; contrary to expectations, invertebrate genomes do not contain sequences belonging to the TAAR family of GPCRs in spite of the fact that trace amine binding has been demonstrated in several invertebrate species including *Drosophila melanogaster* (Saudou *et al*., 1990), honeybee (*Apis mellifera*) (Blenau *et al*., 2000; Grohmann *et al*., 2003) and molluscs (Gerhardt *et al*., 1997). This is in contrast to the extensive inter-species homology reported for adrenoceptors and for muscarinic cholinergic receptors (Venter *et al*., 1984; Pelacios *et al*., 1989).

The extensive analysis of vertebrate and invertebrate genomes (Gloriam *et al*., 2005a, b) leads to the conclusion that all vertebrate TAARs likely are derived from a common ancestor that existed prior to the separaton of fish and mammalian lineages. Additional analysis of their deduced amino-acid sequences allowed Lindemann *et al*. (2005) to separate each species's trace amine receptor gene family into three subdivisions consisting of TAAR1-4, TAAR5 and

Table 1 TAAR classification and nomenclature

	Human		*Chimpanzee*		*Rat*		*Mouse*	
Group 1	TAAR1	TA1	TAAR1		TAAR1	TA1	TAAR1	TA1
	TAAR2	GPR58	(TAAR2)		TAAR2		TAAR2	
	(TAAR3)	(GPR57)	(TAAR3)		TAAR3		TAAR3	
	(TAAR4)	(TA2)	(TAAR4)		TAAR4	TA2	TAAR4	
Group 2	TAAR 5	PNR	TAAR5		TAAR5		TAAR5	
Group 3	TAAR6	TA4	TAAR6	TA4	TAAR6	TA4	TAAR6	
	(TAAR7)		(TAAR7)		TAAR7a		TAAR7a	
					TAAR7b	TA12	(TAAR7b)	
					TAAR7c		TAAR7c	
					TAAR7d	TA15	TAAR7d	
					TAAR7e	TA14	TAAR7e	
					(TAAR7f)	(TA13)	TAAR7f	
					TAAR7g	TA9		
					TAAR7h	TA6		
					(TAAR7i)			
	TAAR8	TA5	(TAAR8)		TAAR8a	TA11	TAAR8a	
					TAAR8b	TA7	TAAR8b	
					TAAR8c	TA10	TAAR8c	
	TAAR9	TA3	(TAAR9)		TAAR9	TA3	TAAR9	

List of TAAR genes in human, chimpanzee, rat and mouse. The table is derived from Lindemann *et al*. (2005). Pseudogenes are shown in brackets. Old names used in previous papers are reported on the right of the new standard name. TA stands for 'trace amine receptor'. Other abbreviations were sometimes used in the place of TA, namely TAR or TRAR. GPR stands for G-protein receptor. PNR stands for putative neurotransmitter receptor. Human TA2 was also named 5-HT4, since it was initially interpreted as a serotonin receptor pseudogene. Human TA5 was also named GPR102.

TAAR6-8. In contrast, invertebrate trace amine-activated receptors are not closely related to vertebrate TAARs but rather more similar to receptors activated by serotonin, in particular 5HT1 receptors. Therefore, it appears that, during speciation, the capability to preferentially bind trace amines has developed at least twice, and that vertebrate TAARs have not evolved from the invertebrate receptors for trace amines.

Subsequent to the reports of Borowsky *et al*. (2001) and Bunzow *et al*. (2001), for a long time, all attempts to heterologously express other putative trace amine receptors proved to be unsuccessful except possibly for TAAR4. Although there have been reports made at scientific meetings claiming expression and characterization of various rat and human trace amine receptor clones, none have been published until the very recent description of the results obtained in HEK293 cells transfected with human or mouse TAARs (Liberles and Buck, 2006). An explanation for the widespread difficulty to heterologously express most TAARs *in vitro* remains elusive.

Several authors reported TAAR transcripts to have a broad tissue distribution. *In situ* hybridization histochemistry revealed the presence of TAAR1 mRNA in many regions of the mouse brain (Borowsky *et al*., 2001). Using quantitative RT-PCR to amplify mRNA isolated from various human tissues, Borowsky *et al*. (2001) found that TAAR1 is expressed at moderate levels (100 copies/ng cDNA) in the stomach and at low levels (15–100 copies/ng cDNA) in the amygdala, kidney, lung and small intestine. Trace amounts were detected in liver, pancreas, prostate, skeletal muscle, spleen, as well as in many central nervous system locations. TAAR6 and TAAR8 were expressed in kidney and amygdala (Borowsky *et al*., 2001), while TAAR9 was expressed in kidney, pituitary and skeletal muscle (Vanti *et al*., 2003). Human leukocytes express TAAR1, TAAR6, TAAR8 and TAAR9 (D'Andrea *et al*., 2003), whereas rat hearts contain transcripts for TAAR8a, and, at a lower level, TAAR1, TAAR2, TAAR3 and TAAR4 (Chiellini G *et al*., unpublished observations).

Recently, Liberles and Buck (2006) reported that all TAAR subtypes, except for TAAR1, are expressed in the mouse olfactory epithelium. Double-labeling experiments suggested that different TAAR subtypes are expressed in different cells, and that TAARs are not coexpressed with odorant receptors. Notably, the authors obtained no evidence for TAAR gene expression in the brain and in other mouse tissues, with a detection threshold of about 100 copies of mRNA per cell. It remains to be determined whether the discrepancy with previous reports is due to species differences or rather due to differences in the sensitivity of the detection technique.

Endogenous TAAR ligands: (a) derivatives of standard amino acids

The metabolites considered by most to be trace amines derive from standard aromatic amino acids (i.e. phenylalanine, tyrosine, tryptophan) through a single enzymatic step: decarboxylation catalyzed by either aromatic L-amino acid decarboxylase (AADC; E.C. 4.1.1.28) or L-histidine decarboxylase (E.C. 4.1.1.22), the former known to be a rather non-selective enzyme since it requires only an aromatic group linked to alanine C(β) as the key feature for substrate recognition (Berry *et al*., 1996). The action of AADC directly yields *p*-tyramine from L-tyrosine, β-phenylethylamine from L-phenylalanine and tryptamine from L-tryptophan. Amino-acid decarboxylase is a single enzyme with one catalytic site but with different locations for attachment of the substrates. The enzyme is widely distributed in the brain and in the peripheral tissues. Recent investigations have shown that the enzyme is regulated by short-term mechanisms that may involve activation of adenyl cyclase or protein kinase C. In addition, a long-term mechanism of activation by altered gene expression has been suggested (Zhu and Juorio, 1995). Interestingly, a specific allele of the human dopamine D2 receptor is associated with increased activity of AADC in the striatum (Laakso *et al*., 2005).

The synthesis of some trace amines requires additional steps. The major biosynthetic route for *m*-tyramine formation is by the hydroxylation of phenylalanine, probably by the enzyme tyrosine hydroxylase to produce *m*-tyrosine, followed by decarboxylation catalyzed by AADC (Dyck *et al*., 1983). Octopamine can be produced from *p*-tyramine by the enzyme dopamine β-hydroxylase. Trace amines can also be synthesized from the conversion of the corresponding secondary amines (*N*-methyltyramine, *N*-methylphenylethylamine, *N*-methyltryptamine and synephrine) by the enzyme phenylethanolamine *N*-methyltransferase or by the nonspecifc enzyme *N*-methyltransferase, which are widely expressed in brain and in peripheral tissues (Saavedra *et al*., 1973, 1974).

Trace amine inactivation primarily involves the oxidation of the amino group, a reaction that is catalyzed by monoamine oxidase (MAO; E.C. 1.4.3.4). There are two MAO isoforms: MAO-A and MAO-B. Each isoform displays a characteristic preference for each trace amine; for example, β-phenylethylamine is primarily oxidized by MAO-B (Yang and Neff, 1973), while the other trace amines are metabolized by both MAO-A and MAO-B (Philips and Boulton, 1979; Durden and Philips, 1980). The occurrence and physiological importance of tryptamine oxidation by polymorphic cytochrome P450 isoenzymes is still a matter of debate (Paterson *et al*., 1990; Yu *et al*., 2003). The rate at which trace amines are metabolized is quite high with half-lives in the order of 30 s (Durden and Philips, 1980). Cellular membranes do not represent a significant barrier to trace amine diffusion and no substantial vesicular storage has been documented in the literature for any of these substances, with the exception of *p*-tyramine which appears to be stored in synaptic vesicles (Berry, 2004 and references therein).

Endogenous trace amine levels have been determined in the central nervous systems of some species of vertebrates and significant differences have been reported to exist between brain regions. In rat brain, the estimated concentration of trace amines in whole tissue ranges: 11–44 nM for β-phenylethylamine, 1–102 nM for *p*-tyramine, 0.4–73 nM for *m*-tyramine, 0.4–8 nM for tryptamine and 7–59 nM for octopamine (Berry, 2004 and references therein). Similar concentrations have been estimated in peripheral tissues (Durden *et al*., 1973; Saavedra and Axelrod, 1973; Philips *et al*., 1974; Kinniburgh and Boyd, 1979; Ibrahim *et al*., 1985;

Durden and Boulton, 1988; D'Andrea *et al*., 2003). In terms of cellular distribution, each radiolabeled trace amine appears to have a rather uniform distribution, with its concentration determined principally by the relative rate of synthesis and degradation.

Although evidence in support of there being specific trace amine-binding sites in vertebrate tissues has been accumulating over the years (Hauger *et al*., 1982; Cascio and Kellar, 1983; Bruning and Rommelspacher, 1984; Vaccari, 1986; Nguyen and Juorio, 1989), the demonstration of specific receptors was only recently forthcoming. Now with the molecular cloning of rat, mouse, rhesus monkey and human TAAR sequences, they should be amenable to characterization by heterologously and stably expressing them in tissue culture (Borowsky *et al*., 2001; Bunzow *et al*., 2001; Miller *et al*., 2005). In COS-7 cells expressing recombinant human TAAR1, cAMP production can be induced by several biogenic amines in the following order of potency: *p*-tyramine > *β*-phenylethylamine > octopamine > dopamine > > tryptamine, histamine, serotonin, noerpinephrine. The EC_{50} for cAMP production averages 214 and 324 nM for *p*-tyramine and *β*-phenylethylamine, respectively, while it is over one order of magnitude higher for octopamine. A similar order of potency is observed in binding studies with regard to [^{3}H]tyramine displacement. Similarly, in HEK293 cells stably expressing that the rat TAAR1 cloned independently by Bunzow *et al*. (2001), the rank order of potency for cAMP production is *p*-tyramine > *β*-phenylethylamine > tryptamine > octopamine > *m*-tyramine > > dopamine. In this cell line, the EC_{50} for *p*-tyramine and *β*-phenylethylamine averages 69 and 240 nM, respectively.

In general, TAAR1 orthologues obtained from different species show EC_{50} values for *p*-tyramine and *β*-phenylethylamine in the range of 0.1–1.4 μM (Lindemann *et al*., 2005; Liberles and Buck, 2006), while EC_{50} values for octopamine and tryptamine fall in the range of 2–10 μM and 1.5–45 μM, respectively (Lindemann *et al*., 2005). Interestingly, secondary amines such as *N*-ethyl-*p*-tyramine, *N*-ethyl-*β*-phenylethylamine and synephrine are slightly more potent than the corresponding primary amines. In contrast, dopamine and serotonin are 5- to 25-fold less potent than *p*-tyramine and *β*-phenylethylamine, respectively, based on EC_{50} values and maximum cAMP levels achieved, which are about half of what the trace amines produce (Lindemann *et al*., 2005), suggesting that they may act as partial agonists. At the rat TAAR1 histamine, adrenaline and noradrenaline at 1 μM concentrations are totally ineffective in stimulating cAMP production in tissue culture cells (Bunzow *et al*., 2001).

Studies performed with COS-7 cells expressing the rat TAAR4, previously known as TA2 (see Table 1), also show stimulation of cAMP production by trace amines, but the effect is limited to *β*-phenylethylamine and *p*-tyramine with EC_{50}'s in the order of 1.9 and 17 μM, respectively (Borowsky *et al*., 2001). The mouse TAAR4 shows EC_{50}'s > 50 μM for all trace amines (Lindemann *et al*., 2005). However, an EC_{50} ~ 1 μM for *β*-phenylethylamine was obtained in HEK293 cells transfected with the mouse TAAR4, if the latter was modified with an amino-terminal addition that facilitates cell-surface expression (Liberles and Buck, 2006).

In contrast, so far no response to *p*-tyramine, *β*-phenylethylamine, tryptamine, octopamine or other biogenic amines has been observed in transfected heterologous cells expressing TAAR subtypes other than TAAR1 or TAAR4 (Borowsky *et al*., 2001; Lindemann *et al*., 2005), and it should be pointed out that TAAR4 is a pseudogene in human. It is, however, worth noting that the apparent refractory nature of other members of this receptor family to trace amine agonism might just as well be an artefact of the heterologous expression systems being employed or second messengers being monitored. Coexpression of rhesus monkey TAAR1 and human dopamine transporter was associated with increased cAMP production by *β*-phenylethylamine and decreased cAMP production by tyramine (Miller *et al*, 2005). It is not clear whether these findings are due to the modulation of amine transport or rather due to the direct interaction between TAAR1 and dopamine transporter.

In conclusion, the identification of physiologically and behaviorally relevant interactions between *p*-tyramine or *β*-phenylethylamine (or other trace amines) and members of the TAAR family remains elusive. So far pharmacological effects were reported only for two receptor subtypes, using trace amines at concentrations, which are substantially higher than their endogenous levels. These considerations have fueled the search for other endogenous ligands.

Endogenous TAAR ligands: (b) thyronamines

An interesting line of research grew out of the extensive structure activity functional profiling of various species of TAAR1 carried out in the Grandy laboratory. The insight these investigators had was to realize that the *β*-phenylethylamine skeleton is an essential molecular feature that all TAAR1 agonists share and that this structural element is present in the decarboxylated skeleton of thyroid hormone molecular derivatives known as thyronamines (Scanlan *et al*., 2004).

Thyroid hormone metabolism involves different types of reactions, namely inner and outer ring deiodinations, deamination/transamination, decarboxylation and esterification (Wu *et al*., 2005). Decarboxylation is usually thought to occur after the amino group has been removed by MAOs or amino transferases, yielding tyroacetic acid derivatives. However, it cannot be excluded that decarboxylation may precede deamination, for example, as it does in the biosynthesis of the catecholamine series of neurotransmitters, and hence thyroxine, 3,5,3′-triiodothyronine, as well as their lower iodination state metabolites are converted to the corresponding arylethylamine compounds, also known as iodothyronamines (Figure 3).

Given the structural similarity between iodothyronamines and other biogenic amines it followed that iodothyronamines could be agonists of TAAR1. So, to test this hypothesis, nine different thyronamines were chemically synthesized (Scanlan *et al*., 2004; Hart *et al*., 2006) and then evaluated in transfected HEK293 cells stably expressing either the rat or mouse TAAR1. This approach revealed that several thyronamine derivatives induced a concentration-dependent

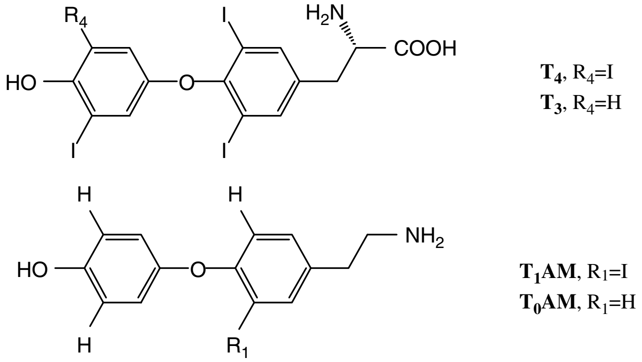

Figure 3 Structures of thyroid hormones and of thyronamines. T_0AM, thyronamine; T_1AM, 3-iodothyronamine; T_3, 3,5,3′-triiodothyronine; T_4, thyroxine.

increase in cAMP concentration. 3-Iodothyronamine (T_1AM) appeared to be the most effective compound, with EC_{50} values in the range 10 to 100 nM for the rat and mouse receptors, respectively. 3,5-Diiodothyronamine, 3,5,3′-triiodothyronamine and the deiodinated derivative thyronamine were also effective, with decreasing EC_{50}. Importantly, T_1AM at concentrations $<10\,\mu$M does not modify cAMP production in HEK293 cells expressing either the D1 dopamine receptor or the β2 adrenoceptor, and it has no affinity for the traditional nuclear thyroid hormone receptors (Scanlan *et al*., 2004) that belong to the nuclear receptor superfamily of hormone-activated transcription factors (Yen, 2001), and conversely 3,5,3′-triiodothyronine does not activate heterologously expressed TAAR1.

The combined use of liquid chromatography and tandem mass spectrometry allowed the identification of thyronamines (particularly, T_1AM) in tissue homogenates derived from the brain and other tissues, confirming that these molecules can be correctly referred to as endogenous compounds. Although the assay was not quantitative, the use of internal standards showed that whole tissue T_1AM content is in the order of 100 pmol/g (Scanlan *et al*., 2004; Chiellini *et al*., unpublished observations). It should be emphasized that the subcellular distribution of thyronamines is unknown. Therefore, their effective concentrations in specific compartments might be significantly different from the average concentrations.

While most effects of thyroid hormone are mediated by changes in gene transcription, non-genomic effects have also been described, which occur within a matter of seconds to minutes and are insensitive to inhibitors of protein synthesis such as cyclohexamide (Davis and Davis, 1996), although the underlying transduction pathways remain obscure (Falkenstein *et al*., 2000). Such effects concern sugar and calcium uptake (Davis and Blas, 1983; Segal and Ingbar, 1989), oxygen consumption (Ikemoto *et al*., 1967), ion channel activation (Yalcin *et al*., 1999) and cardiac function (Davis and Davis, 2002).

Therefore, it was interesting to evaluate the functional consequence of T_1AM exposure, both *in vivo* and *in vitro*, on isolated organs. To date, several effects have been described and, surprisingly, they are opposite in direction to those produced by thyroid hormone, suggesting that T_1AM could employ GPCRs as mediators of short-term modulation. In adult C57BL/6J mice, intraperitoneal injection of either T_1AM or thyronamine (20–50 mg/kg) produced a rapid, dose-dependent decrease in body temperature (Scanlan *et al*., 2004). In isolated rat hearts, infusion of either T_1AM or thyronamine (20–60 μM) produced a rapid, dose-dependent decrease in cardiac output and heart rate, providing evidence of a negative inotropic and chronotropic action (Chiellini *et al*., 2004; Scanlan *et al*., 2004). The decrease in heart rate was also confirmed in the *in vivo* mouse model, after intraperitoneal administration. In all cases, T_1AM appeared to be more potent than thyronamine, and the effectiveness ratio was comparable to that observed in the heterologous cell model.

Further investigations are needed to clarify the receptor subtypes responsible for mediating the effects of T_1AM as well as their physiological relevance. Decreases in body temperature and cardiac function are not consistent with increased cAMP production at the cellular level, raising the possibility that, in some tissues, either TAAR1 activation is not coupled to Gs proteins or T_1AM may interact with other receptor subtypes. In rat, the cardiac effects of T_1AM are remarkably accentuated by the tyrosine kinase inhibitor genistein, while they are dampened by the tyrosine phosphatase inhibitor vanadate (Chiellini *et al*., 2005), suggesting that a crucial step in signal transduction involves changes in the phosphorylation state of tyrosine residues. Evidence of tyrosine de-phosphorylation in cytosolic and microsomal proteins has been observed in rat hearts perfused with T_1AM (Chiellini *et al*., unpublished observations).

Although T_1AM is a novel compound only recently discovered, a few studies with other thyronamine congeners were performed many years ago. In the anaesthetized dog, thyronamine administration produced an increase in heart rate and cardiac inotropic state (Buu-Hoi *et al*., 1966; Buu-Hoi *et al*., 1969; Boissier *et al*., 1973; Cote *et al*., 1974). These effects appeared after a lag of about 10 min, and were remarkably blunted or abolished by adrenergic blockade, suggesting at the time that thyronamine induced catecholamine release. In contrast, after catecholamine depletion and/or adrenergic blockade, thyronamine infusion produced an immediate negative inotropic effect (Cote *et al*., 1974). On the basis of our recent findings (Scanlan *et al*., 2004), the latter might represent a TAAR-mediated response. Other investigations were performed with 3,5,3′-triiodothyronamine, which was reported to inhibit prolactin secretion in cultured pituitary cells (Cody *et al*., 1984). While this effect was attributed to interference with the adrenergic system (Meyer and Hesch, 1983), the involvement of TAARs cannot be excluded.

Recently, many thyronamine derivatives have been synthesized, and they have been comparatively evaluated with regard to their ability to induced hypothermia in mice and cAMP production in HEK cells stably expressing either the mouse or the rat TAAR1s (Hart *et al*., 2006). Some derivatives turn out to be more active than T_1AM; namely, 3-methyl-thyronamine, *N*-methyl-*O*-(*p*-triflouromethyl) benzyl-tyramine, *O*-phenyl-3-iodotyramine and *O*-(*p*-fluoro) phenyl-3-iodotyramine. In general, structural–activity relationship studies have led to the following conclusions: a basic amino group at Cα is required for activity, and monomethylation of the amine can be beneficial; an iodide or methyl substituent at the 3-position of the thyronamine scaffold is optimal for activity; the 4′-OH of thyronamine is not necessary for activity but its removal may render the remaining compound difficult to metabolize and possibly result in impaired clearance.

In summary, there is evidence that T$_1$AM and possibly other thyronamines interact with heterologously expressed TAAR1 and produce functional effects *in vivo*. However, there is still no direct evidence that the functional effects of exogenous T$_1$AM are mediated by TAAR1 (or other TAAR subtypes), nor is there conclusive evidence that endogenous T$_1$AM concentrations are sufficient to determine a physiological response *in vivo*. Therefore, the thesis that thyronamines are endogenous TAAR agonists should still be regarded as an interesting working hypothesis.

Odorous amines as TAAR ligands

The observation that most TAAR subtypes are expressed in the mouse olfactory epithelium induced to investigate whether TAARs may interact with odorants. For this purpose, HEK cells were transfected with vectors encoding for individual mouse or human TAARs, and cAMP production was determined after exposure to a large number (over 300) of odorous compounds (Liberles and Buck, 2006). A positive response was obtained with five mouse TAAR subtypes and 13 specific odorants, all of which were amines. In particular, TAAR3 responded to isoamylamine (EC$_{50}=10\,\mu$M), cyclohexylamine (EC$_{50}=20$–$30\,\mu$M), 2-methylbutamine (EC$_{50}=100\,\mu$M), isobutylamine and 3-(methylthio)-propylamine; TAAR4 responded to β-phenylethylamine (EC$_{50}=1\,\mu$M) and *N*,*N*-dimethyl-2-phenylethylamine; TAAR5 responded to trimethylamine (EC$_{50}=0.3\,\mu$M), dimethylethylamine (EC$_{50}=0.7\,\mu$M) and *N*-methylpiperidine; TAAR7f responded to *N*-methylpiperidine (EC$_{50}=20\,\mu$M). The mouse and human TAAR1 also responded to several volatile amines, although this subtype is not expressed in the olfactory epithelium.

These results induced to speculate that TAAR subtypes other than TAAR1 may represent a second class of chemosensory receptors, at least in mouse. Interestingly, at least three putative TAAR ligands, that is isoamylamine, trimethylamine and β-phenylethylamine, can be detected in mouse urine. Their concentration is related to gender or stress exposure, and isoamylamine acts as a pherormone in mouse (Liberles and Buck, 2006). Therefore, these compounds may be regarded as endogenous ligands, and it has been hypothesized that TAARs might be involved in the behavioral and physiological responses to social cues present in urine.

The transduction pathway(s) activated by TAAR stimulation in olfactory sensory neurons have not been determined, although coexpression of several TAAR subtypes (TAAR2, TAAR6, TAAR7f and TAAR9) with Gα_{olf} proteins has been observed in these cells (Liberles and Buck, 2006).

Pharmacological agents as TAAR1 ligands

Given the structural similarity of amphetamine's molecular structure to that of β-phenylethylamine and *p*-tyramine (Figure 4), Bunzow *et al.* (2001) tested the hypothesis that amphetamine and its congeners would be potent agonists of heterologously expressed TAAR1s. In HEK293 cells stably expressing rat TAAR1, amphetamine stimulated cAMP production with an EC$_{50}$ comparable to that of β-phenylethylamine (210 nM for R-amphetamine and 440 nM for S-amphetamine). Amphetamine derivatives also increased cAMP production with different potency. Methamphetamine, 3,4-methylenedioxymetamphetamine (MDMA, known as ‘Ecstasy’) and the hallucinogenic amphetamine 2-amino, 1-[2,5-dimethoxy-4-iodophenyl]-propane (DOI) were only slightly less potent than amphetamine. *N*-ethylderivatives such as fenfluramine and *N*-ethylamphetamine were substantially less effective, while 4-hydroxyamphetamine turned out to be the most potent rat TAAR1 agonist with an EC$_{50}$ of 51 nM. Amphetamine and MDMA also stimulated cAMP production in HEK293 cells stably expressing the cloned rhesus monkey TAAR1 (Miller *et al.*, 2005).

Bunzow *et al.* (2001) also demonstrated that several widely used ergot alkaloids and ergoline derivatives can potently and efficaciously activate the rat TAAR1 in this heterologous model. Effective compounds included ergometrine, dihydroergotamine, D-lysergic acid diethylamide and the antiparkinsonian agents, bromocriptine and lisuride.

Finally, TAAR1-mediated cAMP production was reported in the presence of nonsubstrate inhibitors of dopamine transporter, namely $1\,\mu$M nomifensine and $10\,\mu$M 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MTPT) (Bunzow *et al*, 2001).

All the drugs mentioned previously have other established molecular targets, so the relevance of their interaction with TAAR1 remains to be determined. However, human TAAR genes are all clustered on the long arm of chromosome 6 in one of the few regions (6q23.2), which is consistently associated with schizophrenia (Cao *et al.*, 1997; Levinson *et al.*, 2000; Schwab *et al.*, 2000; Mowry and Nancarrow, 2001) or bipolar affective disorder (Rice *et al.*, 1997; Ewald

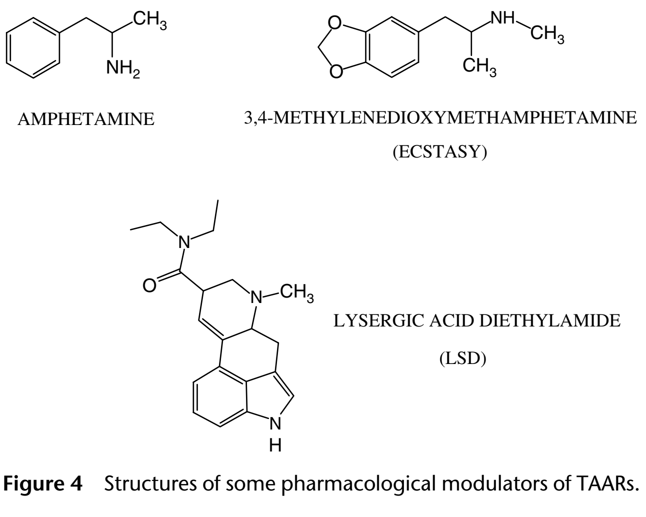

Figure 4 Structures of some pharmacological modulators of TAARs.

et al., 2002; Freudenberg-Hua *et al*., 2003) in linkage studies. Therefore, the finding that psychostimulant and hallucinogenic drugs interact with TAAR1 opens fascinating perspectives about the potential role of this signaling pathway in mental disorders and in drug addiction.

Some molecular genetic studies in recent years have focused on TAARs. A single nucleotide polymorphism in the 3′untranscribed region of TAAR6 gene (previously named as TRAR4) has been associated with susceptibility to schizophrenia in some families of African American or European ancestry (Duan *et al*., 2004), although a statistically significant association was not confirmed in Japanese or Arab Israeli kindreds (Ikeda *et al*., 2005; Amann *et al*., 2006). In another study, a nonsense mutation in the TAAR2 gene has been associated with nearly double the incidence of schizophrenia, although only a small number of patients were genotyped, and the difference versus control did not reach statistical significance (Bly, 2005).

A different single nucleotide polymorphism in the sixth transmembrane domain of TAAR 6 (V265I) was related to bipolar affective disorder in a German pedigree (Abou Jamra *et al*., 2005), although the association was not confirmed in a Swedish population (Venken *et al*., 2006). In contrast, no association was observed between bipolar disorder and a null mutation in TAAR9 (Vanti *et al*., 2003).

Conclusions and perspectives

The discovery of TAARs suggests the existence of novel aminergic system(s) in vertebrates. Trace amines such as tyramine, *β*-phenylethylamine, octopamine and tryptamine are known to play a major role in invertebrate physiology by interacting with specific plasma membrane GPCRs. It appears that in vertebrates TAARs evolved independently from the invertebrate receptors and acquired the ability to interact with different amines, including the decarboxylated thyroid hormone derivatives known as thyronamines, several volatile amines and possibly other as yet unidentified endogenous compounds. The relatively large number of TAAR genes and their allegedly widespread tissue expression suggest that this system has a major physiological importance.

Although the aforementioned picture is attractive, it is worth re-emphasizing that many basic issues are still unresolved. While TAAR gene expression has been observed in several tissues by RT–PCR and *in situ* hybridization, TAAR protein expression has not been formally demonstrated, owing to technical difficulties in developing adequate experimental tools. Effective subtype-specific anti-TAAR antibodies are not yet available, and even the expression of TAARs in heterologous systems has been difficult to achieve, since consistent success has been accomplished only with TAAR1. As a consequence, the best evidence of TAAR-mediated signaling is represented by the pharmacological responses observed in cells expressing TAAR1. Specific binding sites for trace amines and for T_1AM have also been demonstrated, but their molecular identity and subcellular distribution are unknown. The lack of specific TAAR antagonists further complicates the interpretation of pharmacological and radioligand-binding experiments, while transgenic models of TAAR knockout or TAAR overexpression are not available, except for a TAAR1-KO mouse, which was the subject of a preliminary report (Wolinsky *et al*., 2004).

The downstream events involved in TAAR signaling are also poorly understood. Evidence from several laboratories confirms that heterologously expressed TAAR1 can couple with Gs proteins resulting in the stimulation of adenylate cyclase. However, it is possible that different TAAR subtypes might couple with different G proteins, and/or TAAR1 may show different coupling in different cells. In particular, the cardiac effects of thyronamines do not appear to be consistent with increased cAMP, and may involve changes in tyrosine kinase/phosphatase activity.

In spite of these limitations, the potential importance of the new aminergic system(s) should not be overlooked. Modulators of GPCR signaling represent the largest group of drugs currently available. Preliminary evidence that links TAARs to psychiatric diseases and psychotropic agents has been reported, and so exploring and defining the role of TAARs and their ligands in these and other pathological states seems to be the logical next step. Therefore, once TAAR signaling is unraveled and adequate pharmacological tools become available, important new therapeutical opportunities may result.

Conflict of interest

The authors state no conflict of interest.

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