Trace amines: Identification of a family of mammalian G protein-coupled receptors

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Tyramine, β-phenylethylamine, tryptamine, and octopamine are biogenic amines present in trace levels in mammalian nervous systems. Although some ''trace amines'' have clearly defined roles as neurotransmitters in invertebrates, the extent to which they function as true neurotransmitters in vertebrates has remained speculative. Using a degenerate PCR approach, we have identified 15 G protein-coupled receptors (GPCR) from human and rodent tissues. Together with the orphan receptor PNR, these receptors form a subfamily of rhodopsin GPCRs distinct from, but related to the classical biogenic amine receptors. We have demonstrated that two of these receptors bind and/or are activated by trace amines. The cloning of mammalian GPCRs for trace amines supports a role for trace amines as neurotransmitters in vertebrates. Three of the four human receptors from this family are present in the amygdala, possibly linking trace amine receptors to affective disorders. The identification of this family of receptors should rekindle the investigation of the roles of trace amines in mammalian nervous systems and may potentially lead to the development of novel therapeutics for a variety of indications.

Norepinephrine (NE), dopamine (DA), and serotonin (5-HT) are classical biogenic amine neurotransmitters whose well characterized effects are mediated by interactions with subfamilies of receptors that belong to the rhodopsin superfamily of G protein-coupled receptors (GPCRs). In addition to these classical amines, there exists a class of ''trace amines'' that are found in very low levels in mammalian tissues, and include tyramine, β-phenylethylamine (β-PEA), tryptamine, and octopamine (1). The rapid turnover of trace amines, as evidenced by their dramatic increases following treatment with monoamine oxidase (MAO) inhibitors or deletion of the MAO genes, suggests that the levels of trace amines at neuronal synapses may be considerably higher than predicted by steady-state measures (2–5). The role of trace amines as neurotransmitters in invertebrates is well established and octopamine is thought to be the sympathetic nervous system counterpart to NE (6–9). GPCRs for tyramine and octopamine have been cloned from both insects (10–14) and mollusks (15, 16).

Although there is clinical literature that supports a role for trace amines in depression as well as other psychiatric disorders and migraine (2, 3, 17–20), the role of trace amines as neurotransmitters in mammalian systems has not been thoroughly examined. Because they share common structures with the classical amines and can displace other amines from their storage vesicles, trace amines have been referred to as "false transmitters" (21). Thus, many of the effects of trace amines are indirect and are caused by the release of endogenous classical amines. However, there is a growing body of evidence suggesting that trace amines function independently of classical amine transmitters and mediate some of their effects via specific receptors (for review, see refs. 22–24). Saturable, high-affinity binding sites for [^{3}H]tryptamine (23, 25–27), *p*-[^{3}H]tyramine (28–30), and β-[^{3}H]PEA (31) have been reported in rat brain, and both the pharmacology and localization of these sites suggest that they are distinct from the amine transporters. However, although binding sites in brain and other tissues have been reported, no specific receptors for these trace amines have yet been identified conclusively.

We now report the identification of a family of related mammalian GPCRs of which two members have been shown to specifically bind and/or be activated by trace amines. TA_1 is activated most potently by tyramine and β-PEA, and TA_2 is activated most potently by β-PEA. The 15 distinct receptors described here, along with the orphan receptor PNR (32) and the pseudogenes GPR58, GPR57 (33), and the 5-HT_4 pseudogene (34), share a high degree of sequence homology and together form a subfamily of rhodopsin GPCRs distinct from but related to 5-HT, DA, and NE receptors. We further describe the localization of TA_1 in human and rodent tissues, as well as the chromosomal localization of the human members of this family. The identification of this family of receptors should facilitate the understanding of the roles of trace amines in the mammalian nervous system.

Materials and Methods

Degenerate PCR. To clone a rat TA_1 fragment, PCR was performed on genomic DNA by using primers designed based on an alignment of the sixth (5′-TNNKNTGYTGGYTNCCNT-TYTTY-3′) and seventh (5′-ARNSWRTTNVNRTANCC-NARCC-3′) transmembrane (TM) domains of a subset of 5-HT receptors. To clone rat TA_4, human TA_5, rat TA_7, rat TA_8, and rat TA_9, PCR was performed on genomic DNA by using primers designed based on an alignment of the first intracellular loop and TMII (5′-TTYAARCARYTNCAYWSNCCNAC-3′) or the first extracellular loop (5′-GARHVNTGYTGGTAYTTYGG-3′) and TMVI (5′-ATNCCNARNGTYTTNRCNGCYTT-3′ or 5′-CCARCANRNNARRAANACNCC-3′) of TA_1, GPR58, and GPR57. To clone TA_2, PCR was performed on rat genomic DNA by using primers designed based on an alignment of the first intracellular loop TMII (5′-TTYAARSMNYTNCAY-WSNCCNAC-3′) and the first extracellular loop (5′-CCRAARWACCARCANBNYTCNRY-3′) of TA_3, TA_1, GPR58, PNR, and the 5-HT_4 pseudogene. For the cloning of a rat TA_3 fragment, PCR was performed on genomic DNA by using primers designed based on an alignment of TMVI (5′-GYNTWYRYNNTNWSNTGGHTNCC-3′) and TMVII (5′-

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Abbreviations: 5-HT, serotonin; CFTR, cystic fibrosis transmembrane conductance regulator; CNS, central nervous system; DA, dopamine; GPCR, G protein-coupled receptor; MAO, monoamine oxidase; NE, norepinephrine; β-PEA, β-phenylethylamine; TA, trace amine; TM, transmembrane domain.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AF380185–AF380203).

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AVNADNGBRWAVANNANNGGRTT-3′) of a collection of rhodopsin GPCRs. PCR conditions were: 94°C for 5 min; 10 cycles of 94°C for 30 s, 44°C for 45 s or 43°C for 1 min (TA_1), 72°C for 1 min 45 s; 30 cycles of 94°C for 30 s, 49°C for 45 s or 48°C for 1 min (TA_1), 72°C for 1 min 45 s; 72°C for 20 min. PCR products were subcloned into the TA cloning vector (Invitrogen), sequenced (Big Dye cycle sequencing protocol and ABI 377 sequencers from Applied Biosystems), and analyzed (WISCONSIN Package, Genetics Computer Group, Madison, WI).

Library Screening. Rat liver or human placental genomic phage libraries (Stratagene) or a rat cosmid library (CLONTECH) were screened by using ^{32}P-labeled oligonucleotide probes and standard protocols. Positive signals were isolated and hybridizing bands, identified by Southern blot analysis, were subcloned into pcDNA3.1 (Invitrogen) or a modified form of pcEXV (35) and sequenced as above.

Low Stringency PCR. Fragments of species homologues of TA_1 were amplified from genomic DNA using primers designed against the rat TA_1. PCR was performed with the Expand Long Template PCR System (Roche Molecular Biochemicals) with an annealing temperature of 45–51°C.

Rapid Amplification of cDNA Ends (RACE). 5′ and 3′ RACE were performed according to the manufacturer's protocol, using Marathon-Ready cDNA (CLONTECH) from kidney and stomach (human TA_1), kidney and testes (rat TA_2), spinal cord (rat TA_3), and brain (mouse TA_1). Coding regions were amplified multiple times from genomic DNA, human amygdala cDNA, or rat testes cDNA by using primers specific to the 5′ and 3′ untranslated regions.

Oocyte Injection and Recording. Oocytes were isolated from *Xenopus laevis* (Xenopus 1, Ann Arbor, MI) and maintained, injected, incubated, and recorded from as described (36). Oocytes were injected with 10–15 ng of mRNA encoding TA_1 with or without 10 ng of mRNA encoding the cystic fibrosis transmembrane conductance regulator (CFTR; ref. 37). Ligands were applied by local perfusion from a 10-μl glass capillary tube 0.5 mm from the oocyte.

Measurement of Intracellular cAMP. Transiently transfected COS-7 cells were incubated in Dulbecco's PBS supplemented with 10 mM Hepes, 10 mM glucose, 5 mM theophylline, and 10 μM pargyline for 20 min at 37°C in 95% O_2/5% CO_2. Test compounds were added and cells were incubated for 10 min. The medium was aspirated and the reaction stopped by the addition of 200 μl of 100-mM HCl. The cAMP content in each well was measured by RIA (Scintillation Proximity Assay; Amersham Pharmacia Biotech) using a microbeta Trilux counter (Wallac, Gaithersburg, MD).

Radioligand Binding. Membranes prepared from cells transiently transfected with human TA_1 and rat $G\alpha_s$ were diluted in 25 mM Gly-Gly buffer (Sigma, pH 7.4 at 0°C) containing 5 mM ascorbate (final protein concentration = 120 μg/ml). Membranes were then incubated with [^{3}H]tyramine [American Radiochemicals, St. Louis; specific activity 60 mCi/μM (1 Ci = 37 GBq)] in the presence or absence of competing ligands on ice for 30 min in a volume of 250 μl. Bound ligand was separated from free ligand by filtration through GF/B filters presoaked in 0.5% polyethyleneimine, using a Brandel (Bethesda, MD) cell harvester vacuum filtration device, and bound radioactivity quantified by using a scintillation counter. Data were fit to nonlinear curves by using PRISM (GraphPad, San Diego).

Quantitative Reverse Transcription (RT)–PCR. cDNA was prepared from DNase-treated total RNA purchased from CLONTECH or isolated from human tissues by using TRIzol reagent (Life Technologies, Grand Island, NY). Integrity of RNA and cDNA was assessed by amplification of cyclophilin or glyceraldehyde-3-phosphate dehydrogenase (GAPDH). PCR reactions were carried out in a PE7700 sequence detection system (Perkin–Elmer) according to the manufacture's protocol. The probe [5′(6-FAM)-ATGGTGAGATCTGCTGAGCACTGTTGG-TATT-(TAMRA)3′] was labeled with FAM (6-carboxyfluorescein) as the reporter and TAMRA (6-carboxy-4,7,2,7′-tetramethylrhodamine) as a quencher, and the forward and reverse PCR primers were 5′-CATGGCCACTGTGGACTT-TCT-3′ and 5′-GTCGGTGCTTGTGTGAATTTTACA-3′, respectively. The fluorescent signal from each well was normalized by using an internal passive reference, and data were fitted to a standard curve generated with genomic DNA.

Chromosomal Localization. The Stanford Human Genome Center (SHGC) G3 panel of 83 radiation hybrids was analyzed by PCR using 20 ng of DNA and the same primers, probes, and thermal cycler profiles as used for localization. The RH Server (at SHGC; www-SHGC.stanford.edu) and the National Center for Biotechnology Information's LocusLink and GeneMap '99 were used for analysis.

***In Situ* Hybridization Histochemistry.** Sense and antisense riboprobes (251 bp, TMV-TMVI of mouse TA_1) were labeled with digoxigenin as outlined in the DIG/Genius System (Roche Molecular Biochemicals). Male 129S6/SVEV mice (20 g, Taconic Farms) were anesthetized with ketamine 20 mg/kg (Research Biochemicals) and xylazine 0.2 mg/kg (Sigma), and perfused transcardially with PBS followed by 4% paraformaldehyde/PBS. Tissues were cryoprotected, stored at −20°C, and sectioned (30 μM) by using a freezing microtome. Free-floating sections were incubated in 100 mM glycine for 5 min and 0.3% Triton X-100 for 15 min, then rinsed twice in PBS for 5 min. *In situ* hybridization histochemistry was carried out on free-floating tissue sections as outlined in the DIG/Genius System with a hybridization temperature of 52°C in a buffer containing 40% formamide.

Results

In an attempt to identify additional 5-HT_1-like receptors, such as the elusive 5-HT1p receptor (38), degenerate PCR primers were designed against TMs VI and VII of an alignment of 5-HT_1 receptors and used to amplify rat genomic DNA at reduced stringency. One product from this reaction was found to be a DNA sequence, not found in the GenBank database, with 42–48% amino acid identity to 5-HT_4, DA D_2, and β-adrenergic receptors. Sequencing of the corresponding full-length cDNA, BO111, revealed an ORF of 996 bp that is predicted to encode a protein of 332 aa (Fig. 1, rat TA_1). An allelic variant of this receptor was also identified wherein a glutamine replaces a leucine at position 170. BO111 is most closely related to GPR58 (50% aa identity), the human 5-HT_4 pseudogene (47% aa identity, with frame shifts "corrected"), BO107 (an orphan GPCR previously identified at Synaptic and later renamed TA_3) and GPR57 (45% aa identities), PNR (38% aa identity), and 5-HT_{1D}, 5-HT_4, and 5-HT_7 receptors (35–37% aa identities). Human and mouse orthologues of BO111 were obtained by standard methods. The amino acid sequences of the human and mouse receptors are 76% identical to each other and 79% and 87% identical to the rat receptor, respectively (Fig. 1).

A search for the endogenous ligand for the receptor encoded by BO111 was performed by expressing it in oocytes along with mRNA encoding the cAMP-responsive Cl channel, CFTR. Candidate ligands were tested in eleven groups of five. From this broad panel, octopamine and, more weakly, DA and 5-HT, elicited inward currents at 100 μM (Fig. 2*A*). Stimulation by

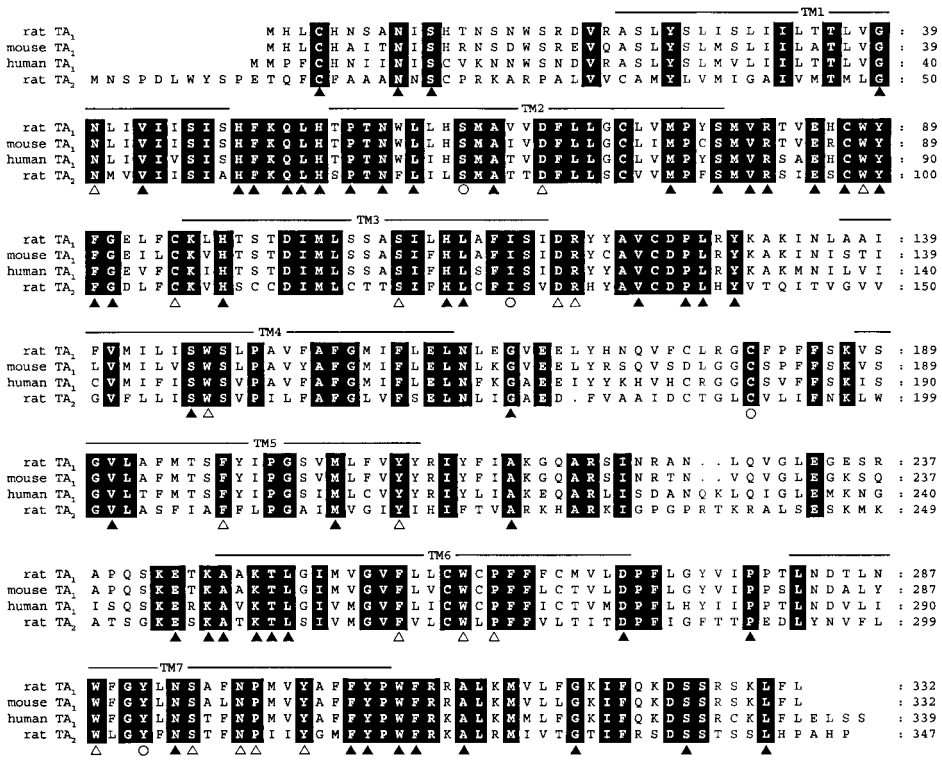

Fig. 1. Alignment of rat, mouse, and human TA_1 and rat TA_2 receptors (GenBank accession nos. AF380186, AF380187, AF380185, and AF380188, respectively). Shaded residues are conserved in all four receptors. Triangles and circles indicate residues conserved in TA_1–TA_{15}. Open triangles are also conserved among all human monaminergic receptors, and open circles are conserved among all human 5-HT but not NE or DA receptors. Seven putative TM domains are indicated.

octopamine (100 μM) produced an average current amplitude of 230 ± 55 nA (n = 4). Similar currents were generated by tyramine at a lower concentration (100 nM; 287 ± 31 nA, n = 28; Fig. 2*B*). EC_{50} values were obtained for octopamine (635 ± 151 nM) and tyramine (37 ± 4.4 nM) from cumulative concentration effect responses (data not shown). These results suggested that BO111 encodes a receptor for trace amines, and was thus named TA_1. No such currents were observed in oocytes injected with only mRNA encoding the CFTR channel. Oocytes expressing rat TA_1 without CFTR failed to generate inward currents (Fig. 2*C*; n = 11), suggesting that stimulation of rat TA_1 by octopamine and tyramine resulted in the generation of cAMP leading to CFTR channel opening, presumably via activation of the endogenous *Xenopus* G protein $G\alpha_s$. Oocytes expressing the human orthologue of rat TA_1 with CFTR also produced inward currents in response to application of 100 nM tyramine (Fig. 2*D*).

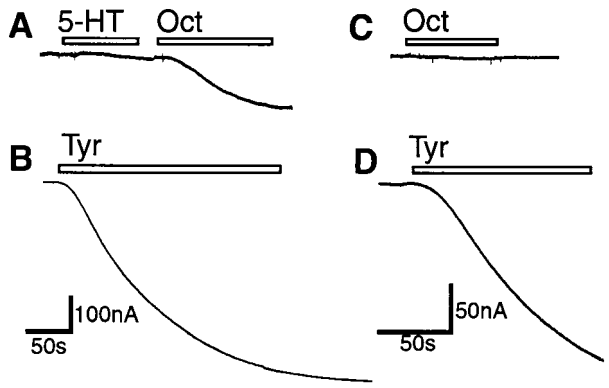

Fig. 2. Voltage-clamp responses to trace amines in oocytes. (*A*) Response to 100 μM octopamine (Oct) or 5-HT in an oocyte expressing rat TA_1 and CFTR. (*B*) Response to 100 nM tyramine (Tyr) in an oocyte expressing rat TA_1 and CFTR. (*C*) Response to 100 μM octopamine in an oocyte expressing only TA_1. (*D*) Response to 100 nM tyramine (Tyr) in an oocyte expressing human TA_1 and CFTR. Holding potential was −80 mV for all oocytes. Marker bar in *D* also applies to *A* and *C*.

Additional bioamines were tested for activity at human TA_1 expressed in mammalian cells. Human TA_1 was activated most potently by β-PEA and tyramine, and more weakly by octopamine and DA (Table 1 and Fig. 3). The agonists listed in Table 1 produced an increase in intracellular cAMP accumulation, likely via the $G\alpha_s$-class of G proteins in COS-7 cells transfected with human TA_1, but not in mock-transfected cells.

Consistent with the relatively high potency of tyramine for activating human TA_1, [^{3}H]tyramine demonstrated high-affinity, saturable binding in TA_1-expressing membranes (average K_d = 20 nM; data not shown). Selectivity of human TA_1 for β-PEA

Table 1. Pharmacological profile of human TA_1

Compound	K_i, nM	EC_{50}, nM
β-PEA	8.0 ± 3.2	324 ± 110
Tyramine	34 ± 11	214 ± 67
Dopamine	422 ± 11	6,700 ± 1,700
Octopamine	493 ± 99	4,029 ± 75
Tryptamine	1,084 ± 159	>6 uM
Histamine	3,107 ± 1,593	>5 uM
Serotonin	>6 uM	>10 uM
Norepinephrine	>10 uM	>5 uM

Values represent the average ± SEM from $n \geq 3$ experiments. Binding K_is were determined from displacement of [^{3}H]tyramine (20 nM) and EC_{50} values were determined by increases in cAMP accumulation.

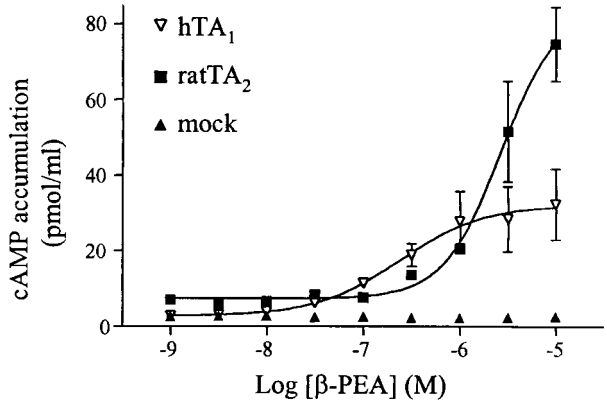

Fig. 3. β-PEA-induced responses in COS-7 cells transfected with human TA_1, rat TA_2, or vector. Cells were incubated with increasing concentrations of β-PEA and cAMP accumulation measured. Data are from duplicate determinations and are representative of three to six experiments.

and tyramine is shown in Table 1. The rank order of potency was similar between the binding and functional assays, although the K_i values determined from binding displacement were ≈6-fold lower than EC_{50} values determined in functional studies. This difference has been reported for many exogenously expressed receptors, including the 5-HT_7 receptor (39), and may be due to the relatively low expression levels of TA_1 in COS-7 cells, weak coupling of the receptor to signaling components in these cells, or differences in assay conditions.

Additional degenerate PCR work performed on rat genomic DNA led to the identification of TA_2. TA_2 is most similar to the human 5-HT_4 pseudogene (82% aa identity with frame shifts "corrected"), and shares 48–51% aa identity to the rat and human TA_1 receptors (Fig. 1), GPR57 and GPR58. The expression of rat TA_2 in COS-7 cells resulted in an increase in cAMP accumulation, presumably via $G\alpha_s$-class G protein(s). Of the biogenic amines tested, only β-PEA and tryptamine activated this receptor; however, the response was of low potency [EC_{50} = 1.9 ± 0.5 μM (Fig. 3) and 17 ± 2 μM (data not shown) respectively]. The low potency of trace amines for rat TA_2 in heterologous expression systems may be explained by its poor surface expression, as determined by subcellular localization of an epitope-tagged rat TA_2 (data not shown). Alternatively, other more potent agonists may exist for rat TA_2. Because the human orthologue of this receptor is most likely the 5-HT_4 pseudogene, no further studies were conducted on rat TA_2.

Further degenerate PCR work led to the identification of TA_5 from human genomic DNA, and TA_4, TA_7, TA_8, and TA_9 from rat genomic DNA. While isolating these full-length receptors from genomic libraries, several additional closely related receptors were also isolated, including human TA_4 and rat TA_6, TA_{10}, TA_{11}, TA_{12}, TA_{13}, TA_{14}, and TA_{15}. TA_4–TA_{15} are highly homologous to each other, with overall aa identities of 62–96% (see Fig. 6, which is published as supplemental data on the PNAS web site, www.pnas.org). These receptors are 66–73% identical to TA_3, 41–48% identical to TA_2, 40–44% identical to TA_1, and 28–36% identical to 5-HT receptors. As indicated in Fig. 1, there are 74 residues that are completely conserved in TA_1–TA_{15}. Of these, 52 are uniquely conserved in the trace amine family (closed triangles), 18 (of 25) are also conserved in all human monoaminergic receptors (open triangles), and an additional 4 (of 32) are conserved with human 5-HT receptors, but not NE or DA receptors (open circles).

A phylogenetic tree was constructed from the aa sequences of TA_1–TA_{15}, PNR, GPR57, GPR58, the 5-HT_4 pseudogene, and several vertebrate and invertebrate aminergic receptors (Fig. 4).

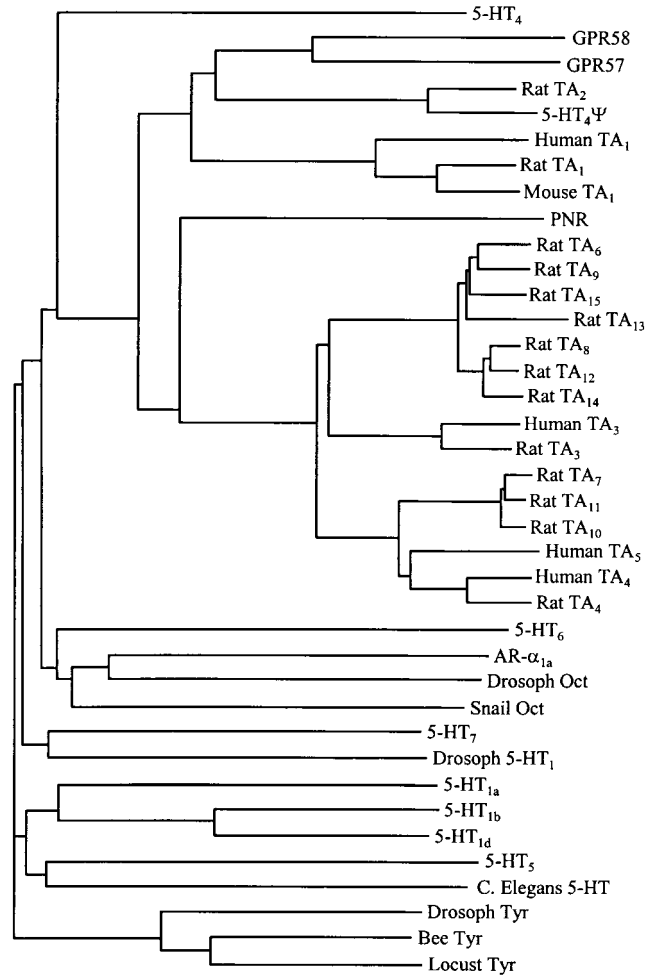

Fig. 4. A phylogenetic tree for trace amine receptors TA_1–TA_{15}, human 5-HT receptors 5-HT_{1a}, 5-HT_{1b}, 5-HT_{1d}, 5-HT_4, 5-HT_5, 5-HT_7, human α1a receptor (AR-α_{1a}), GPR57, GPR58, PNR, 5-HT_4 psuedogene (5-$HT_{4\psi}$), drosophila (Drosoph) receptors for octopamine (Oct), 5-HT_1 and tyramine (Tyr), *Caenorhabditis elegans* (C. Elegans) 5-HT receptor, tyramine receptors from bee (Bee Tyr) and locust (Locust Tyr), and a snail octopamine receptor (Snail Oct). Amino acid sequences for each receptor spanning from the start of TMI to the end of TMVII were aligned by using the CLUSTALW algorithm and the tree constructed by the NJ method on a DecypherII Bioaccelerator (TimeLogic, Reno, NV). GenBank accession nos.: AF380190 (rat TA_3), AF380189 (human TA_3), AF380191 (rat TA_4), AF380192 (human TA_4), AF380193-AF380203 (TA_5–TA_{15}).

The TA receptors, along with GPR57, GPR58, 5-HT_4 pseudogene, and PNR, branch separately from mammalian receptors for classical biogenic amines, including those for 5-HT, and from the invertebrate trace amine receptors. Within this large family of receptors, there appears to be at least two subfamilies. TA_1 and TA_2, along with GPR57, GPR58, and 5-HT_4 pseudogene, constitute one subfamily, and TA_3–TA_{15} constitute a second subfamily.

Radiation hybrid mapping using primers selective for TA_1, TA_3, TA_4, and TA_5 was used to identify the chromosomal localization of the human trace amine receptors. All four genes had virtually identical patterns and mapped to SHGC-1836. This placed the TA receptor genes in the region of chromosome 6q23.2. Interestingly, PNR, GPR57, GPR58, and 5-HT_4 pseudogene were previously shown to be clustered between 6q22 and 6q24 by using fluorescence *in situ* hybridization analysis (32, 33).

Human TA_1 mRNA was detected by quantitative reverse transcription (RT)–PCR in low levels in discrete regions within the central nervous system (CNS) and in several peripheral

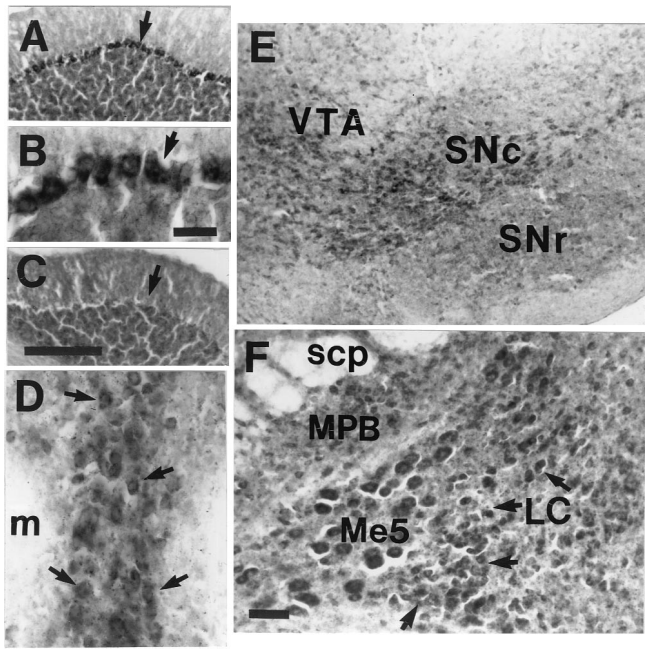

Fig. 5. Photomicrographs showing hybridization signals for TA_1 mRNA in mouse CNS. Signal detected in cerebellar Purkinje cells (arrows) hybridized with antisense (*A* and *B*) and sense (*C*) probes. Scale bar in *C* (200 μm) also applies to *A* and *E*; scale bar in *B*, 25 μm. Photomicrographs showing hybridization signal in cells (arrows) in the dorsal raphe (*D*), ventral tegmental area (VTA), substantia nigra, compact part (SNc) and reticular part (SNr) (*E*), and locus coeruleus (LC; *F*). Scale bar in *F* (50 μm) also applies to *D*. MPB, medial parabrachial nucleus; Me5, mesencephalic trigeminal nucleus; m, medial longitudinal fasciculus; scp, superior cerebral peduncle.

tissues. Moderate levels (100 copies/ng cDNA) were expressed in stomach, low levels (15–100) expressed in amygdala, kidney, lung, and small intestine, whereas trace amounts (<15) were expressed in cerebellum, dorsal root ganglia, hippocampus, hypothalamus, liver, medulla, pancreas, pituitary, pontine reticular formation, prostate, skeletal muscle, and spleen. Message levels for the other human trace amine receptors were also detected in low levels. TA_3 mRNA was detected only in kidney. TA_4 and TA_5 mRNA were expressed in kidney and amygdala, and TA_4 was also detected in the hippocampus.

A widespread and unique distribution of TA_1 mRNA was revealed in the mouse CNS by *in situ* hybridization histochemistry (Fig. 5) and the hybridization signal was localized to the cytoplasm of neuronal profiles (Fig. 5 *A* and *B*). Several brain regions exhibited intense labeling specifically, the mitral cell layer of the olfactory bulb, piriform cortex, the arcuate, motor, and mesencephalic trigeminal nuclei, lateral reticular and hypoglossal nuclei, cerebellar Purkinje cells, and ventral horn of the spinal cord. Moderate labeling was evident in the frontal, entorhinal, and agranular cortices, the ventral pallidum, thalamus, hippocampus, several hypothalamic nuclei, ambiguus, dorsal raphe, and gigantocellular reticular nuclei. Weaker staining was visible in the septum, basal ganglia, amygdala, myelencephalon, and spinal cord dorsal horn. Particularly interesting was the moderate expression of TA_1 mRNA in several monoaminergic cell groups, namely the dorsal raphe (Fig. 5*D*), the locus coeruleus (Fig. 5*F*), and the ventral tegmental area (Fig. 5*E*).

Discussion

We have identified a multigene family of intronless GPCRs and have demonstrated that TA_1 is potently activated by tyramine and β-PEA and displays low affinity for tryptamine, octopamine, and DA. An additional member of this family, TA_2, is also activated by β-PEA and tryptamine. Although the roles of tyramine and octopamine as neurotransmitters acting via stimulation of G protein-coupled receptors in invertebrate systems are well established (6–9), mammalian GPCRs for trace amines have not, to our knowledge, been previously reported. The present finding lends strong support to a role for trace amines as neurotransmitters or neuromodulators in vertebrates.

One of the most interesting and unexpected findings of this study was the discovery of such a large family of highly related receptors. In addition to TA_1 and TA_2, we have identified 13 other related receptors. These additional receptors, TA_3–TA_{15}, share an unusually high degree of amino acid identity (62–96%). For comparison, the human 5-HT receptors share 28–63% amino acid identities. The high degree of homology within members of the TA family and the tight clustering of the human TA receptors on chromosome 6q23.2 suggests that these receptors evolved relatively recently, after the invertebrate/vertebrate split, and makes it tempting to speculate that this region may represent a hotspot for gene duplication events.

Although the degree of homology between receptors within a species is extremely high, the degree of amino acid identity among orthologues is moderate to low. The rat and human orthologues of TA_3 and TA_4 share a moderate degree of amino acid identities (87% and 88%, respectively). However, the mouse, rat, and human orthologues of TA_1 share a relatively low degree of homology (87% for rat and mouse, 79% for rat and human, 76% mouse and human). This observation suggests that although these receptors are relatively recent expansions of the genome, they are evolving at a rapid rate.

Another interesting observation is that a larger number of rat receptors has been identified so far as compared with human receptors. Four human receptors have been identified (TA_1, TA_3, TA_4, and TA_5), whereas 14 rat receptors have been identified (TA_1–TA_4 and TA_6–TA_{15}). To date, only a human form of PNR has been reported (29). There are also a large number of pseudogenes within the human members of this family. The $5\text{-}HT_4$ pseudogene (31), which we propose should be renamed ψTA_2, and ψGPR57 (33) each contain frame shifts resulting in premature stop codons, whereas ψGPR58 lacks an amino terminus (33). Although additional human receptors may ultimately be identified, the striking difference in the number of rat and human receptors suggests that this family may play very different roles in different species.

We have demonstrated a functional response to heterologously expressed TA_1 in both *Xenopus* oocytes and a mammalian cell system. The response in both assays indicates that TA_1 couples to the stimulation of adenylate cyclase through a $G\alpha_s$ G protein. The human TA_1 receptor is activated by tyramine and β-PEA, less potently by octopamine, and binds β-PEA and tyramine with high affinity and tryptamine, octopamine, and DA with lower affinity. The rat TA_2 receptor is activated by β-PEA and tryptamine, also via stimulation of a $G\alpha_s$ G protein. Thus far, we have not demonstrated functional responses to tyramine, β-PEA, tryptamine, octopamine, or the classical biogenic amines in COS-7 cells expressing TA_3–TA_{15}. This finding may be due to poor trafficking to the plasma membrane (data not shown), or these receptors may respond to related, perhaps as yet unidentified, amines. However, the high degree of sequence conservation between the two subfamilies, the evolutionary branching analysis, as well as the chromosomal proximity of the receptors make it very likely that TA_3–TA_{15} encode receptors for trace amines.

Human TA_1 mRNA is expressed in low to moderate levels in peripheral tissues such as stomach, kidney, and lung, and within the CNS appears to be restricted primarily to the amygdala. The expression of TA_1 mRNA is lower than that seen for receptors of classical neurotransmitters, consistent with the low levels of trace amines relative to other neurotransmitters. All of the human members of the TA family are expressed in the kidney, supporting

a role in blood pressure regulation and electrolyte homeostasis. This may be related to the "cheese effect," wherein dietary-induced elevations in tyramine levels in patients taking MAO inhibitors results in hypertension and migraine (see ref. 19 for review).

The expression of TA_1 mRNA in human amygdala is intriguing in light of evidence suggesting a role of trace amines in the etiology and/or treatment of depression and anxiety disorders. A functional deficiency of β-PEA and tryptamine has been proposed as a potential etiological factor in depression (40–42), and increased levels of β-PEA are associated with the manic phase of bipolar disease (43). Antidepressants that inhibit MAO produce proportionally greater increases in trace amines than 5-HT (2, 3). MAO-B knockout mice have increased levels of β-PEA (7-fold higher), but normal levels of 5-HT, NE, and DA (5). Interestingly, MAO-B knockout mice show a reduced decrease in mobility in the forced swim test, similar to that induced by antidepressants (5). Taken together, these results suggest that TA_1 receptors in the amygdala may be an important site of action for trace amines, particularly β-PEA, in the etiology and treatment of depression. The expression of mouse TA_1 mRNA in the dorsal raphe, locus ceruleus, and ventral tegmental area indicates that trace amines may modulate the activity of 5-HT, NE, and DA systems and further supports a role for trace amine receptors in the regulation of mood.

Human trace amine receptor genes map to chromosome 6q23.2, close to SCZD5, a susceptibility locus for schizophrenia (6q13-26 with the greatest allele sharing at 6q21-22.3; ref. 44). Because of structural and physiological similarities, β-PEA has been described as the body's endogenous amphetamine (45, 46). Amphetamine produces a paranoid schizophrenic syndrome in humans, and chronic treatment with either amphetamine or β-PEA produces a behavioral sensitization in animals (47–51). Moreover, numerous clinical studies have demonstrated elevated urinary levels of β-PEA in schizophrenic patients (52, 53). Thus, it will be important to delineate the role of TA receptors in the etiology and treatment of schizophrenia.

Although trace amines have long been thought to be neurotransmitters, the understanding of their physiology has lagged that of the classical biogenic amines, in part, because the receptor targets remained elusive. The identification of mammalian GPCRs for trace amines supports a role for trace amines as bona fide neurotransmitters in vertebrates. The localization of mRNA for three of the four human receptors in amygdala lends a potential site of action for the postulated role of trace amines in the etiology and/or treatment of several affective disorders. Future characterization of TA_3–TA_{15} will further enhance our understanding of these receptors. The discovery of this family of receptors provides a means to evaluate the physiological roles of trace amines in higher species and their regulation in diseased processes, and to explore potential therapeutic applications associated with these receptors.

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