Commentary

Following the trace of elusive amines

Richard T. Premont, Raul R. Gainetdinov, and Marc G. Caron*

Howard Hughes Medical Institute, Departments of Cell Biology and Medicine, Duke University Medical Center, Durham, NC 27710

The classical biogenic amines (serotonin, noradrenaline, dopamine, and histamine) play important roles as neuromodulators. These transmitters are synthesized from precursor amino acids in specific neurons and stored in vesicles at synaptic terminals for release into the synaptic cleft in response to neuronal depolarization. In the extracellular space, the released amines bind to specific receptor proteins—primarily of the G protein-coupled receptor family—on both presynaptic and postsynaptic cells, where they modulate intracellular second messenger pathways to alter the signaling of the fast neurotransmitter-gated ion channels. After release, amines are taken back rapidly into the presynaptic neurons through specific transporters for repackaging into vesicles and re-release or are degraded to inactive products.

The neurochemistry of biogenic amines is relatively well understood, including the control of amine synthesis from precursor amino acids, their storage and release, and their reuptake versus degradation. Imbalances in the levels of these amine neuromodulators are thought to underlie altered brain function in many pathological conditions, including dystonias, Parkinson's disease, schizophrenia, drug addiction, and mood disorders. This obvious involvement of biogenic amines in multiple brain disorders has led to many years of effort to understand their action and to therapeutic interventions to correct deficits, either through activating or inhibiting the synthesis, storage, signaling, or metabolism of individual amines.

For several decades, however, neurochemists and pharmacologists have appreciated that in addition to the major amine neuromodulators a series of less well characterized amines derived from the metabolism of amino acids are also present in many tissues in the body, but especially in the brain (1–4). A recent study published by Borowsky *et al.* in PNAS (5) is certain to rekindle the interest in this class of compounds. These amines include tyramine, tryptamine, octopamine, and β-phenylethylamine (1). In invertebrates, which lack the noradrenaline system, octopamine appears to serve as a major neurotransmitter/neuromodulator (6). In mammals, however, these so-called trace amines are present at generally low levels and there do not appear to be dedicated synapses using exclusively any of the trace amines (1–4, 6). Nevertheless, levels of these amines are altered in various disorders (Table 1), and blockade of amine degradation leads to significant accumulations of trace amines indicative of a high level of synthesis and turnover, suggesting that these trace amines may play important roles. One of the roles suggested for these compounds is as "false transmitters," which displace biologically active biogenic amines from their storage and act on transporters much like the amphetamines (7). However, these compounds are not thought of as active neuromodulators.

Results from the study (5) suggest that the trace amines may be much more than metabolic curiosities or aminergic wannabe's, but may function as distinct and bona fide neuromodulators. Using degenerate amplification, bioinformatics and comparative genomics, Borowsky and coworkers have identified 15 members of two distinct families of G protein-coupled receptors with a high degree of similarity to traditional G protein-coupled biogenic amine receptors. They demonstrate that one of these receptors, called TA1, is a receptor for two of the trace amines, β-phenylethylamine and tyramine. TA1 binds to both β-phenylethylamine and tyramine with high affinity and produces cAMP in response to this binding, whereas the related TA2 receptor appears to be specific for β-phenylethylamine and tryptamine. Both of these G protein-coupled receptor families possess many of the structural hallmarks of the rhodopsin/β-adrenergic receptor superfamily (8). Among these are several highly conserved stretches of residues in the predicted transmembrane (TM) regions, such as the (E/D) R (Y/H) motif at the end of the third TM domain and the NPXXY motif in the seventh TM, as well as potential sites of regulatory phosphorylation in the C-terminal domain. Thus, these receptors are likely to couple to conventional signaling pathways as demonstrated for TA1 (5) and their signaling is likely to be regulated through mechanisms similar to those for other G protein-coupled receptors (8). Several of these new receptors are expressed within specific regions of the central nervous system, whereas others appear to be found in specific peripheral tissues such as stomach, kidney, lung, and small intestine. In the central nervous system, the mRNA for the TA1 and TA2 receptor proteins can be found sparsely expressed in certain cells of the substantia nigra/ventral tegmental area, locus coeruleus, and dorsal raphe nucleus, areas where the cell bodies for the classic biogenic amines neurons are found (5).

Table 1. Potential trace amines dysregulation in human disorders (1–4)

β-Phenylethylamine	Phenylketonuria, migraine, schizophrenia, depression, attention deficit/hyperactivity disorder
Tyramine	Migraine, hypertension, schizophrenia, Parkinsonism, depression
Octopamine	Hypertension, hepatic encephalopathy, phenylketonuria, depression
Tryptamine	Depression, schizophrenia, hepatic encephalopathy

That G protein-coupled receptors exist in the brain that respond specifically to trace amines such as β-phenylethylamine and tyramine satisfies one additional criterion for classifying these molecules as neurotransmitters/neuromodulators. Although there appear to be no neurons using any of the trace amines exclusively, these molecules can be packaged and released along with traditional amines (1–4, 7), and so may function as traditional neuromodulators working through their own receptors. In addition, because these trace amines are primarily generated through decarboxylation of their respective amino acid precursors via aromatic amino acid decarboxylase (1–4), which is involved in the synthesis of the major monoamine neurotransmitters, a spatially restricted mechanism for their synthesis exists. Levels of trace amines also seem to be dynamically regulated, because inhibition of monoamine oxidase leads to

See companion article on page 8966 in issue 16 of volume 98.

*To whom reprint requests should be addressed. E-mail: caron002@mc.duke.edu.

www.pnas.org/cgi/doi/10.1073/pnas.181356198

marked elevation of certain of these substances (1–3). It is known that diet rich in these amines such as cheese (tyramine) and chocolate (β-phenylethylamine) can increase blood pressure and cause migraine specifically in patients taking monoamine oxidase inhibitors (9). Thus, although additional studies will be necessary to unambiguously classify these molecules as neurotransmitters, the study by Borowsky *et al.* (5) represents a major step in this direction. It is worth mentioning that the status of histamine as a neurotransmitter was similarly controversial for some time after its initial discovery (10).

Members of the second, apparently distinct, TA3 receptor family remain uncharacterized. The high degree of similarity with both the TA1 tyramine/β-phenylethylamine receptor family and with traditional biogenic amine receptors strongly suggests that these TA3-class receptors likewise recognize trace amines or other endogenous small molecules derived from amino acids. Other candidate amine ligands for these receptors include other derivatives of phenylethylamines (such as phenylethanolamine) as well as derivatives of indoleamines (such as 5-methoxytryptamine) that are also present in trace amounts in the body (1–3). These novel receptors may possibly bind other nonaminergic endogenous compounds such as γ-hydroxybutyric acid, a drug of abuse commonly referred to as "the date rape drug" for which the mechanisms of action remains unknown (11). Further studies should clarify which endogenous ligands these receptors recognize. This TA3 family appears quite large in rodents, but substantially smaller in humans. The reason for this diversity and variation among species remains unknown, but may reflect differing levels of redundancy and/or gene duplication.

Classical pharamacological studies on trace amines like β-phenylethylamine have suggested that these compounds may function primarily as "endogenous amphetamines" or "false neurotransmitters" (1–4). Identification of specific receptors for these amines establishes a mechanism by which these compounds can lead to specific effects of their own, rather than simply interfering with the actions of classical neuromodulators. The fact that the transcripts for these receptors appear to be widely expressed in many areas of the brain, but particularly in the monoaminergic cell groups and amygdala, suggests involvement of these amines in the regulation of cognition, emotion, and affect. It is interesting to note that in *Drosophila*, tyramine synthesis by the enzyme tyrosine decarboxylase is required for the development of sensitization to cocaine. Exposure of fruit flies to cocaine leads to an increase in tyrosine decarboxylase activity, which parallels the development of sensitization (12). It will be very interesting to examine the potential role of these amines in mechanisms of sensitization and reward in mammals.

It is known that diet rich in these amines such as cheese and chocolate can increase blood pressure and cause migraine specifically in patients taking monoamine oxidase inhibitors.

Because the trace amines have been known to be altered in brain disorders and have now been shown to bind to G protein-coupled receptors that are highly related to traditional biogenic amine receptors, it will be critical to determine the extent to which drugs used in the treatment of psychiatric diseases actually target these new receptors in addition to their accepted targets. Table 1 summarizes pathological conditions in which the levels of various trace amines have been reported to be altered. A careful analysis of the distribution of individual trace amine receptors also may suggest specific neural circuits where the molecules are key neuromodulators. Moreover the disruption of individual receptor genes may lead to specific behavioral alterations that highlight the function of these intriguing molecules in the normal brain. As the signaling pathways and neural circuits for these receptors are elucidated, these new receptors may represent novel therapeutic targets.

The discovery of a large family of receptors that may function to sense the levels and hence the actions of compounds that were heretofore generally regarded only as trace metabolites illustrates a rapidly broadening concept of biology. That is, organisms seem to have evolved a mechanism to sense and respond not only to major products of synthetic pathways but also to certain trace metabolites and degradation products. Elegant examples of this principle also have been observed with the discovery of cognate ligands for several orphan members of the nuclear receptor superfamily, where many ligands have been found to be not steroids but rather metabolic intermediates of cholesterol and sterol metabolism (13).

1. Usdin, E. & Sandler, M., eds. (1976) *Trace Amines and the Brain* (Dekker, New York), pp. 21–40.
2. Boulton, A. A., Juorio, A. V. & Downer, R. G. H., eds. (1988) *Trace Amines: Comparative and Clinical Neurobiology (Experimental and Clinical Neuroscience)* (Humana, Totowa, NJ).
3. Saaverda, J. M. (1989) in *Catecholamines II*, eds. Trendelenburg, U. & Weiner, N. (Springer, Berlin), pp. 181–201.
4. Janssen, P. A., Leysen, J. E., Megens, A. A. & Awouters, F. H. (1999) *Int. J. Neuropsychopharmacol.* **2,** 229–240.
5. Borowsky, B., Adham, N., Jones, K. A., Raddatz, R., Artymyshyn, R., Ogozalek K. L., Durkin, M. M., Lakhlani, P. P., Bonini, J. A., Pathirana, S., *et al.* (2001) *Proc. Natl. Acad. Sci. USA* **98,** 8966–8971. (First Published July 17, 2001; 10.1073/pnas.151105198)
6. Axelrod, J. & Saavedra, J. M. (1977) *Nature (London)* **265,** 501–504.
7. Parker, E. M. & Cubeddu, L. X. (1988) *J. Pharmacol. Exp. Ther.* **245,** 199–210.
8. Watson, S. & Arkinstall, S., eds. (1994) *The GProtein Linked Receptor Fact Book* (Academic, London).
9. Sandler, M., Youdim, M. B. & Hanington, E. (1974) *Nature (London)* **250,** 335–337.
10. Schwartz, J. C. (1979) *Life Sci.* **25,** 895–912.
11. O'Connell, T., Kaye, L. & Plosay, J. J., 3rd (2000) *Am. Fam. Physician* **62,** 2478–2483.
12. McClung, C. & Hirsh, J. (1999) *Curr. Biol.* **9,** 853–860.
13. Giguere, V. (1999) *Endocr. Rev.* **20,** 689–725.