

PERSPECTIVE

Old Drugs Learn New Tricks: Insights from Mammalian Trace Amine Receptors

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Biogenic amines are important neuromodulators whose discovery laid the foundation of modern neuropharmacology. These compounds (which include norepinephrine, dopamine, serotonin, and histamine) are stored in secretory vesicles located in the cytoplasm, are released into the extracellular space by regulated exocytosis, then activate specific G protein-coupled receptors (GPCRs) located in pre- and postsynaptic plasma membranes of target neurons. The synthesis of biogenic amines from precursor amino acids involves a series of enzymatic reactions and active transport of the products of these reactions into regulated secretory vesicles. Once they have undergone exocytosis, biogenic amines are cleared rapidly from the extracellular space, both by uptake into the cytoplasm via distinct amine transporters located in the plasma membrane and by enzymatic metabolism occurring in the extracellular space and cytoplasm (Cooper et al., 1986; Nestler et al., 2001).

Many of the enzymes, transporters, and receptors involved in the complex biology of biogenic amines have been characterized in great detail, suggesting the possibility that there is little left to be learned in this oldest area of neuropharmacology. Two recent studies, one reported in *Proceedings of the National Academy of Sciences of the United States of America* (Borowsky et al., 2001) and another in this issue of *Molecular Pharmacology* (Bunzow et al., 2001), demonstrate clearly that this is not the case. These studies identify a new family of GPCRs that are potently activated by a subset of so-called "trace amines". Trace amines refer to a number of additional products of amino acid metabolism, as well as intermediates in the synthesis of "classical" biogenic amines, that are present in relatively large amount in neural tissue. Trace amines have been of interest for many years (Usdin et al., 1976; Boulton, 1985), particularly because substantial alterations in the amount of trace amines present in neural tissue are associated with pathological conditions (O'Reilly and

Davis, 1994). However, the physiological effects of trace amines have remained obscure, as noted by Borowsky et al. (2001) and discussed in additional detail in a commentary accompanying that article (Premont et al., 2001). Thus the discovery of mammalian trace amine receptors by Borowsky et al. (2001) and Bunzow et al. (2001) is of fundamental physiological interest. The study by Bunzow et al. (2001) is of particular interest to pharmacologists because it suggests that trace amine receptors may sense major extracellular metabolites of the classical catecholamines dopamine, norepinephrine, and epinephrine. Moreover, the results of Bunzow et al. (2001) suggest that an old and extremely important class of therapeutic and abused drugs, the amphetamines [and related psychostimulant drugs, such as 3,4-methylenedioxymethamphetamine ("ecstasy")], can directly activate trace amine receptors.

Borowsky et al. (2001) and Bunzow et al. (2001) used different strategies, applying degenerate polymerase chain reaction to identify candidate GPCRs sharing sequence homology with serotonin or catecholamine receptors, respectively. These approaches led to the isolation of genomic and cDNA clones encoding a putative GPCR that was called the TA1 receptor because, when expressed in heterologous cells (in which coupling to G_s was observed), this receptor was activated with nanomolar potency by certain trace amines (tyramine, β -phenylethylamine, and tryptamine) but not by "classical" biogenic amines such as dopamine and serotonin. Borowsky et al. (2001) performed reduced stringency screening and phylogenetic analysis suggesting that the TA1 receptor represents one member of two related subfamilies of mammalian GPCRs. Therefore, considerable subtype diversity may exist in this class of GPCRs. The mapping of trace amine receptor genes near a previously proposed susceptibility locus for schizophrenia (Cao et al., 1997), as noted by both groups, has interesting implications that merit further study.

Bunzow et al. (2001) perform extensive pharmacological studies of the TA1 receptor and establish two particularly interesting features of this GPCR. First, the TA1 receptor is activated with higher potency and efficacy by *meta-O*-methyl metabolites of dopamine, norepinephrine, and epinephrine than by the precursor catecholamines themselves. Thus, it is possible that trace amine receptors function physiologically to detect the major extracellular metabolites of classical catecholamines produced by catechol-*O*-methyl transferase. These metabolites are altered significantly in cerebrospinal fluid samples collected from patients suffering from certain psychiatric disorders, an observation that has in the past been difficult to link to any specific physiological consequence (Usdin et al., 1976). A second intriguing observation from the studies of Bunzow et al. (2001) is that TA1 receptors can be directly activated by amphetamines and certain related psychostimulant drugs. Experiments leading to this observation were motivated by the fact that amphetamine is structurally quite similar to endogenously produced trace amines (such as β -phenylethylamine) that are TA1 receptor agonists. The ability of these drugs to directly activate TA1 receptors was shown definitively by ligand binding and adenylyl cyclase activation assays conducted on transfected cells expressing recombinant TA1 receptors.

To appreciate the potential significance of this latter observation, it is useful to briefly review our previous understanding of amphetamine's pharmacological effects in the context of the actions of other important drugs that influence signaling by biogenic amines. A wide variety of psychopharmaceuticals modulate neural function by targeting specific steps in the metabolism, transport, or receptor binding of biogenic amines (Nestler et al., 2001). For example, many antidepressant drugs inhibit plasma membrane transporters for serotonin or norepinephrine, causing increased concentrations of these amines in the extracellular space. Certain other antidepressants cause a similar effect via a different mechanism, by inhibiting the enzymatic metabolism of biogenic amines by monoamine oxidase. These "indirect" actions on activation of biogenic amine receptors are in contrast to "direct" effects of drugs that bind to GPCRs themselves. Examples of such substances include many antipsychotic drugs, which are competitive antagonists (or inverse agonists) of D2 dopamine receptors and certain other GPCRs activated by biogenic amines (Thaker and Carpenter, 2001).

According to this classification, amphetamines have been shown definitively to mediate "indirect" effects on GPCR signaling. Amphetamines and other psychostimulants increase extracellular dopamine concentrations by competing for uptake of this catecholamine by plasma membrane dopamine transporters, inhibiting catecholamine uptake by vesicular monoamine transporters, and potentially functioning as "false" neurotransmitters to displace intracellular pools of catecholamine by promoting reversal of transporter function. Gene knock-out studies confirm important roles of both plasma membrane and vesicular amine transporters in mediating the physiological actions of amphetamines but also suggest the existence of remarkable complexity in the in vivo effects of psychostimulant drugs (Uhl et al., 1996; Fon et al., 1997; Wang et al., 1997). Indeed, at present it does not seem that all of the physiological actions of psychostimulants can be ascribed to any single effect on amine transport. In this context, the results of Bunzow et al. (2001), which suggest an

additional role of amphetamines as direct agonists of trace amine receptors, are particularly intriguing.

Nevertheless, it is important to note that there is presently no "smoking gun" to implicate either trace amine receptors or direct agonist activity on these receptors in mediating the in vivo effects of psychostimulant drugs in mammals. Studies of *Drosophila melanogaster* suggest that the trace amine tyramine plays an essential role in cocaine-induced behavioral sensitization, although this study supports an indirect agonist action of cocaine rather than a direct agonist effect on tyramine receptors themselves (McClung and Hirsh, 1999). Certain trace amines are substrates for transporters used by classical catecholamines in mammals, suggesting that psychostimulants could act as indirect agonists of trace amine receptors in a similar manner. Moreover, the observation of Bunzow et al. (2001) that mammalian trace amine receptors are activated by *meta-O*-methylated metabolites of dopamine and norepinephrine suggests another mechanism by which psychostimulants could function as indirect agonists of trace amine receptors, via the production of *O*-methylated metabolites from the increased amounts of extracellular dopamine or norepinephrine induced by these drugs. Both Borowsky et al. (2001) and Bunzow et al. (2001), observed that the TA1 receptor resides in transfected cells primarily in an intracellular compartment. This suggests the additional, although highly speculative, possibility that trace amine receptors may be targets for intracellular (presumably intravesicular) ligands.

In summary, the discovery of mammalian trace amine receptors should motivate vigorous investigation of potentially new mechanisms of psychotropic drug action and receptor cell biology. It is even conceivable that the development of drugs specifically influencing trace amine receptor function could be useful in the therapy of important neuropsychiatric disorders such as depression, attention deficit hyperactivity disorder, and drug addiction. Thus, the discovery of mammalian trace amine receptors and the demonstration of a direct agonist effect of certain psychostimulant drugs are exciting developments on several fronts, which suggest that (perhaps) old drugs *can* learn new tricks.

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