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Commentary

Serotonin receptor knockouts: A moody subject

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The neurotransmitter serotonin (5-hydroxytryptamine; 5-HT) is believed to play a significant role in determining one's emotional state. Indeed, serotonergic synapses are sites of action for a number of mood-altering drugs, including the now-legendary antidepressant Prozac (fluoxetine) (1). As a result, there has been tremendous interest in identifying molecular components of the serotonergic system, including cell surface receptors and transporters, and understanding whether and how these proteins contribute to the regulation of mood and emotion. This quest is driven, in part, by the possibility that behavioral disorders, such as depression or anxiety, may be linked to deficits in one or more components of this signaling system. Such information could, in turn, focus attention on specific targets for the development of novel drugs with which to treat psychiatric disorders. In the case of serotonin, this is a particularly challenging goal because the system is quite complex, consisting of at least 14 distinct receptor subtypes (2). Nevertheless, pharmacological and physiological studies have highlighted a subset of 5-HT receptor subtypes worthy of more immediate genetic analysis. One of these, the 5-HT_{1A} receptor, is the focus of two studies by Ramboz *et al.* (3) and Heisler *et al.* (4) in recent issues of the *Proceedings*. These groups used gene "knockout" methods to generate mouse lines lacking 5-HT_{1A} receptors so that they could assess the effects of receptor ablation on behavior, using models of anxiety and depression.

Why is the 5-HT_{1A} subtype considered to be a particularly important and interesting member of the serotonin receptor family? One reason is that the 5-HT_{1A} receptor has for years been synonymous with the classical "autoreceptor" on serotonergic neurons in raphé nuclei of the brain stem (5). These cells synthesize the majority of serotonin in the brain, sending their axonal projections throughout the central nervous system. Activation of 5-HT_{1A} receptors on cell bodies of these neurons inhibits release of serotonin, thereby attenuating serotonergic signaling at large. As such, this receptor represents a potentially important regulatory site for modulating the actions of serotonin in the brain and spinal cord. Interest in this receptor also stems from clinical success with drugs that interact with this site. Most notably, partial agonists of the 5-HT_{1A} receptor, such as buspirone and gepirone, are effective as anxiolytic (antianxiety) agents (6, 7), suggesting that alterations in 5-HT_{1A} receptor activity may be linked to serotonin-mediated changes in mood. In addition, antagonists of the 5-HT_{1A} receptor appear to accelerate and enhance the antidepressant action of so-called selective serotonin reuptake inhibitors (SSRIs) such as Prozac (8, 9), which increase levels of serotonin in the synaptic cleft by blocking transporters on the presynaptic membrane. In other words, blockade of inhibitory autoreceptors may augment the ability of SSRIs to elevate synaptic serotonin levels. Related speculation suggests that desensitization of 5-HT_{1A} autoreceptors represents a significant component of the antidepressant action of chronically administered SSRIs (10). Finally, it must be mentioned that not all 5-HT_{1A} receptors are presynaptic; postsynaptic receptors are expressed in a number of brain regions to which serotonergic neurons project, including the hippocampus, cerebral cortex, and amygdala (11, 12). As in the case of presynaptic autoreceptors, activation of postsynaptic 5-HT_{1A} receptors leads to hyperpolarization of the neuron and the consequent inhibition of neurotransmitter release. This effect appears to be mediated through a biochemical signaling pathway in which 5-HT_{1A} receptors activate a G protein (G_i)-coupled inwardly rectifying potassium channel (13, 14).

In light of the pharmacological evidence that 5-HT_{1A} receptors exert negative "feedback" control on serotonergic neurons, one would predict that mice lacking this receptor should show elevated levels of extraneuronal serotonin, or an increase in the amount of serotonin released after nerve stimulation. However, neither group observed a significant change in the serotonin content of brains from mutant animals compared with wild-type controls. Furthermore, Ramboz *et al.* (3) measured the amounts of serotonin released after electrical stimulation of slices taken from mesencephalic and hippocampal regions of the brain. In slices from wild-type animals, the 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) reduced serotonin release by 30–40%, suggesting that at least some component of autoregulation via the 5-HT_{1A} receptor is reconstituted in this assay. Interestingly, knockout animals showed no significant difference from wild-type controls in the amount of electrically evoked serotonin release from these slices. Moreover, in contrast to wild types, the evoked release observed in mutants was unaffected by pretreatment with 8-OH-DPAT, as one would expect in the absence of functional 5-HT_{1A} receptors. If one assumes that the *in vitro* slice preparation recapitulates regulation by presynaptic autoreceptors *in vivo* (perhaps a large assumption because somatodendritic receptors are lost in this preparation), then these findings suggest either that the 5-HT_{1A} receptor does not play a significant part in modulating serotonin release or that its role has been subsumed by another subtype.

Perhaps the most obvious candidate for such a functional substitution is the 5-HT_{1B} receptor. Like the 5-HT_{1A} receptor, the 5-HT_{1B} subtype is coupled negatively to adenylate cyclase and can be found on serotonergic cells of the raphé nucleus, where it is located at the axon terminal (2, 5). 5-HT_{1B} agonists, such as the antimigraine drug sumatriptan, can inhibit neurotransmitter release from these cells, as Ramboz *et al.* demonstrate, using slices from wild-type or mutant brains. The authors suggest that 5-HT_{1B} receptors may be up-regulated in the brains of knockout mice to compensate for the loss of somatodendritic 5-HT_{1A} autoreceptors, thereby modulating the release of serotonin (and possibly other transmitters) through increased inhibition at the axon terminal. If so, then the serotonergic feedback circuit shows impressive plasticity in the face of a genetic mutation that might otherwise perturb homeostatic mechanisms controlling transmitter release. These findings also remind us that the effects of any given

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mutation or chronic drug treatment on behavior may involve changes at the cellular or molecular level that are several steps removed from their initial point of action. In any case, the model of 5-HT_{1B} receptor compensation put forth by Ramboz *et al.* is still quite hypothetical and must be put to more stringent tests. René Hen's group has generated 5-HT_{1B} receptor knockout mice (15), and crosses with the 5-HT_{1A} receptor knockout line could produce some interesting animals with which to carry out double-mutant analysis of feedback control mechanisms within the serotonergic pathway.

On the behavioral level, the thrust of these two papers is to examine the connection between the 5-HT_{1A} receptor and anxiety, and to validate the use of these mutant mice as a model system for studying anxiety-related mood disorders. As mentioned above, at least part of the motivation for this work comes from the fact that 5-HT_{1A} agonists are prescribed as anxiolytics, and antagonists may facilitate the antidepressant actions of SSRIs. To examine the effects of 5-HT_{1A} receptor ablation on anxiety states, both groups compare the behaviors of wild-type, heterozygous, and mutant mice in various paradigms that measure an animal's willingness to explore open spaces versus its propensity to remain near walls or within enclosed zones. Mice that shun open spaces and prefer to be in covered areas are considered to be more anxious than animals with greater exploratory activity—in essence, an uptight versus relaxed mouse. Heisler *et al.* (4) also compared the willingness of mice to explore a novel object (in this case a table tennis ball) as a means of assessing relative levels of anxiety. Indeed, such assays are validated by the fact that they serve as reasonable predictors of anxiolytic drug efficacy.

What is gratifying about these studies is that both come to essentially the same conclusion even though some of the behavioral assays differ in design and the mouse lines used differ in their genetic backgrounds. In each case, homozygous mutant mice showed less exploratory behavior than wild-type mice. Ramboz *et al.* (3) found some effects of gender in certain exploratory tests wherein only 5-HT_{1A} receptor mutant males exhibited significantly decreased exploratory activity, but this difference seems difficult to interpret as it may be specific to the exact design and sensitivity of a given paradigm. In any case, data from each study indicate that 5-HT_{1A} receptor-deficient animals are, indeed, more anxious than wild-type mice. Both groups also examine wild-type and knockout mice in models of depression. In these assays, the animal is placed in a situation that induces a state of helplessness or behavioral despair, such as being forced to swim or being suspended by the tail. When the animal realizes that it cannot escape, it assumes an immobile position. Antidepressant drugs reduce this period of immobility, and any factor that has the same effect is therefore considered to be antidepressant in nature. Indeed, 5-HT_{1A} receptor mutant mice exhibited substantially shorter immobility periods, especially in the tail suspension assay, supporting the idea that lack of functional 5-HT_{1A} receptors favors a less depressed state, at least under these adverse conditions.

If pharmacological studies have already implicated the 5-HT_{1A} receptor in the modulation of anxiety- and depression-related mood states, then what does the knockout mouse add to our knowledge? As Heisler *et al.* point out, buspirone and other 5-HT_{1A} agonists produce variable results as anxiolytics in different rodent behavioral models. Thus, one important contribution of the present studies is that they provide genetic confirmation of the pharmacological data. Does the genetics tell us anything about the mechanism whereby lack of 5-HT_{1A} receptors heightens anxiety and favors a "less depressed" state? Not yet, because the best guesses (e.g., increased serotonergic activity resulting from loss of presynaptic autoreceptor function) are based largely on models that evolved from earlier pharmacological studies. Moreover, the fact that electrically evoked serotonin release is not altered in brain slices from mutant animals does not rule out autoreceptor-based models, as it is possible that the activity of serotonergic neurons differs in knockout versus wild-type mice under some behavioral circumstances. Thus, to address questions of mechanism it will be necessary, as Heisler *et al.* suggest, to carry out electrophysiological and microdialysis studies in attempts to correlate levels of serotonergic activity with behavior. Also, as Ramboz *et al.* suggest, issues regarding pre- versus postsynaptic functions for 5-HT_{1A} receptors might be clarified by tissue-specific knockouts (16) in which the receptor gene is inactivated solely in cells of the raphé nucleus. Similarly, the interpretation of a standard gene knockout experiment is often complicated by possibilities of long-term developmental changes, a sticking point that can be addressed only by inducible knockout strategies (17) that enable one to eliminate protein expression acutely. For now, Ramboz *et al.* show nicely that administration of a 5-HT_{1A} receptor antagonist "phenocopies" the knockout in the open field anxiety test, lending some weight to the idea that the anxiolytic phenotype does not reflect some long-term developmental change that is several steps removed from receptor inactivation. Nonetheless, these authors have also postulated that compensatory changes do occur at the serotonergic synapse, so the issue is by no means black and white.

Whatever the mechanism, these studies provide yet another example of how a single gene mutation can alter behavior. Is the 5-HT_{1A} receptor knockout mouse a valid model for mood disorders in humans? As Ramboz *et al.* (3) point out, the phenotypes of the knockout mouse seem paradoxical to most people's experience, since heightened anxiety is most often associated with depression. But until we know more about the mechanisms underlying these phenotypes, such comparisons may be difficult to interpret. In the end, the most significant question may be whether behavioral changes in these mice will be good predictors of anxiolytic drug activity in humans. If so, then 5-HT_{1A} receptor knockout mice may earn their keep as sentinels for new therapeutic compounds.

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