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PERSPECTIVE

Why Mice Are Neither Miniature Humans nor Small Rats: A Cautionary Tale Involving 5-Hydroxytryptamine-6 Serotonin Receptor Species Variants

VINCENT SETOLA and BRYAN L. ROTH

Departments of Biochemistry, Psychiatry, and Neurosciences, Case Western Reserve University Medical School, Cleveland, Ohio

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Serotonin [5-hydroxytryptamine (5-HT)] is a biogenic amine neurotransmitter that modulates a host of important biological processes, such as mood, cognition, perception, feeding behavior, smooth muscle contractility, and platelet aggregation. The myriad actions of 5-HT are mediated by one or more of at least seven subtypes of receptors, all but one of which (i.e., the 5-HT_3 receptor) are members of the G protein-coupled receptor superfamily (Kroeze et al., 2002) (see phylogram, Fig. 1). Receptors that are closer on the phylogram are more likely to share pharmacological properties than receptors that are farther apart (distant relatives). One of the most enigmatic 5-HT receptors, the 5-HT_6 receptor, was cloned in 1993 from a rat striatal cDNA library taken from sequence homology with existing 5-HT receptors and found to be expressed in the striatum, olfactory tubercule, cortex, and hippocampus (Monsma et al., 1993; Ruat et al., 1993; Ward et al., 1995). The human (Kohen et al., 1996) and mouse (Kohen et al., 2001) 5-HT_6 receptors were subsequently cloned with the human version shown to be pharmacologically similar to the rat (Kohen et al., 1996). Shortly after the discovery of 5-HT_6 receptors, Roth et al. (1994) reported that a large number of typical and atypical antipsychotic drugs bound with unexpectedly high affinity (K_i values of <20 nM) to cloned 5-HT_6 receptors. The expression of 5-HT_6 receptors in limbic areas and the basal ganglia, and the selective labeling of 5-HT_6 receptors in rat brain with [^{3}H]clozapine (Glatt et al., 1995), strongly implicated 5-HT_6 receptors in at least some of the actions of antipsychotic drugs.

More recently, 5-HT_6 receptors have been demonstrated to regulate central cholinergic neurotransmission. In this regard, the administration of the 5-HT_6 receptor-selective antagonist Ro 04-6790 reversed scopolamine-induced rotation in 6-hydroxydopamine–lesioned rats (Bourson et al., 1998). Additionally, Rogers and Hagan (2001) and Woolley et al. (2001) found that either 5-HT_6 receptor antisense oligonucleotides or 5-HT_6 receptor-selective inhibitors enhanced retention by rats of the learned platform position in the Morris water maze. These data suggest that 5-HT_6 receptor antagonists might boost cholinergic neurotransmission and reduce the cognitive impairments experienced by patients with dementia or schizophrenia. Intriguingly, Tsai et al. (1999) determined that the 267C allele of the 5-HT_6 receptor is a significant risk factor for Alzheimer's disease. Taken together, these findings indicate that 5-HT_6 antagonists might prove useful in treating a number of common illnesses, including dementia and schizophrenia.

The now-classic approach for validating 5-HT_6 receptors as molecular targets for therapeutics is to construct a 5-HT_6 knockout mouse and to characterize its phenotype. As Hirst et al. (2003) discovered, however, it is unlikely that 5-HT_6 knockout mice will be useful for validating the 5-HT_6 receptor as a therapeutic target because of pronounced and unexpected species differences in both receptor regional distribution and pharmacology. It is now widely appreciated that slight differences in rodent and human amino acid sequences can lead to unexpectedly large differences in the pharmacology of the receptors, with potentially disastrous effects for drug-discovery efforts. What has not been clearly documented until the Hirst et al. study (2003), however, is that mouse receptors could be significantly different from rat receptors.

In the article published in this issue of *Molecular Pharmacology*, Hirst et al. (2003) elegantly demonstrate that the mouse 5-HT_6 receptor is, in nearly every respect, distinct from rat and human 5-HT_6 receptors. They investigated these differences because of preliminary studies in which they were unable to quantify mouse 5-HT_6 receptors with a highly selective radioligand ([^{125}I]SB-258585) that labels both rat and human 5-HT_6 receptors. In addition to species differences in the binding of drugs to 5-HT_6 receptors, they found differences in the regional expression of 5-HT_6 receptors. Thus, quantitative polymerase

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; Ro 04-6790, 4-amino-*N*-(2,6 bis-methylamino-pyrimidin-4-yl)-benzene sulfonamide; SB-258585, 4-iodo-*N*-[4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-benzenesulfonamide.

chain reaction studies demonstrated that the mouse 5-HT_6 receptor mRNA was at least 10-fold less abundant than the rat or human 5-HT_6 receptor mRNAs in every brain region examined. Surprisingly, whereas 5-HT_6 receptor mRNA and radioligand binding activity was enriched in the basal ganglia of rat and human brain, there was no such enrichment in the mouse brain.

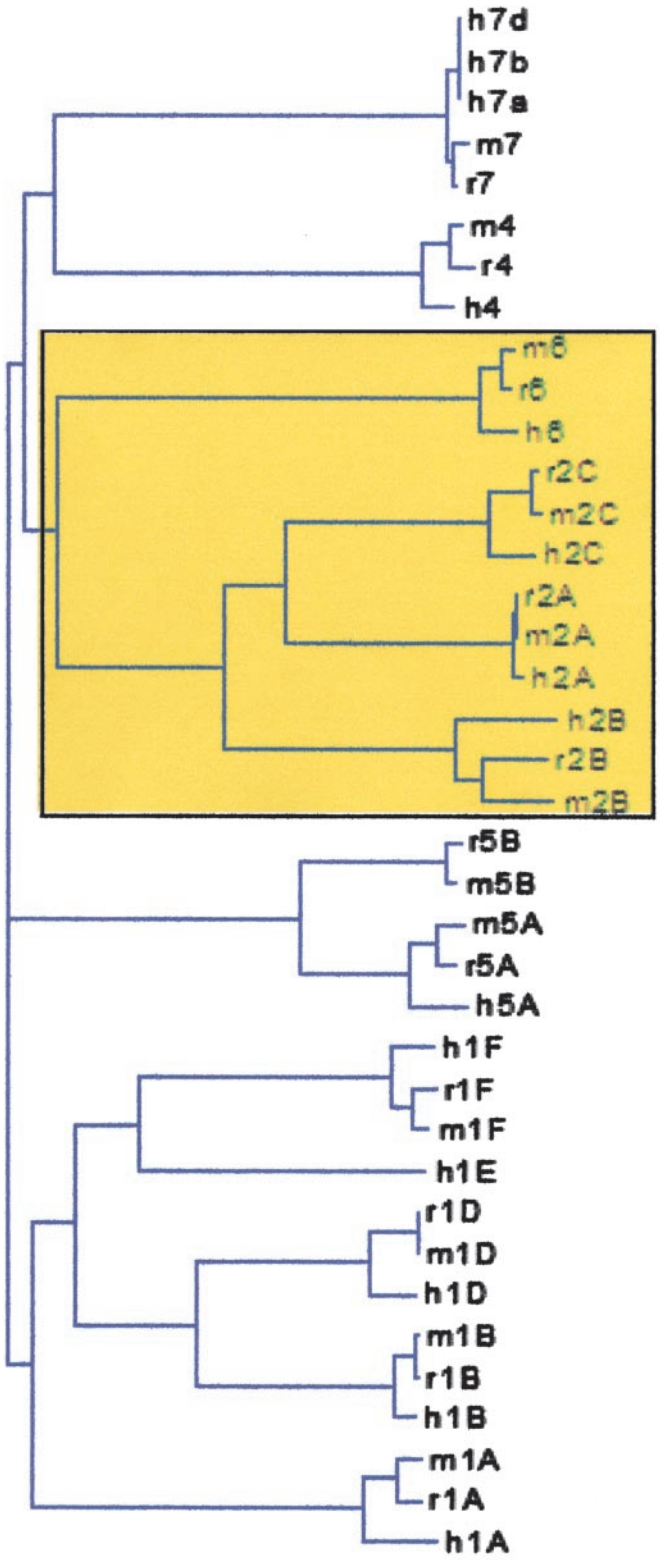

Fig. 1. Phylogram of mouse, rat, and human 5-HT G protein-coupled receptors shows that 5-HT_6 receptors are most similar in amino acid sequence to 5-HT_2 family receptors. Multiple pairwise alignments were performed, and the phylogram was produced using the AlignX module of the Vector NTI Suite (Informax, Frederick, MD).

Additionally, via a combination of site-directed mutagenesis and molecular modeling studies, Hirst et al. (2003) describe the presumed molecular and atomic reasons for the peculiar mouse 5-HT_6 pharmacology. Two amino acids—Tyr188 (in helix 5, which is Phe188 in rats and humans) and Ser290 (in helix 6 which is Asn290 in rats and humans)—were found to account for the bulk of the differences in pharmacology. A nice feature of the study is the parallel inclusion of elegant modeling studies of the various ligands used. Hirst et al. (2003) use this model to present a plausible molecular rationale for the differential interactions of various 5-HT_6 receptor-selective ligands with human, rat, and mouse 5-HT_6 receptors.

The findings described by Hirst et al. (2003) have important implications for drug discovery. Because the mouse 5-HT_6 receptor is distinct *in nearly every way* from the human (and rat) 5-HT_6 receptor, the results force us to question the use of knockout mice in a wholesale fashion to provide validated molecular targets for drug discovery. Their studies strongly imply that before a knockout mouse is accepted as a validated model for a particular human disease, the molecular target needs to be demonstrated to have a pharmacology, regional tissue distribution, and abundance similar to the human homolog. Therefore, this study stands as an important reminder to us all that mice are not miniature humans and, sometimes, not even small rats.

References

Bourson A, Boess FG, Bos M, and Sleight AJ (1998) Involvement of 5-HT6 receptors in nigro-striatal function in rodents. *Br J Pharmacol* **125:**1562–1566.

Glatt CE, Snowman AM, Sibley DR, and Snyder SH (1995) Clozapine: selective labeling of sites resembling 5HT6 serotonin receptors may reflect psychoactive profile. *Mol Med* **1:**398–406.

Hirst WD, Abrahamsen B, Blaney FE, Calver AR, Aloj L, Price GW, Medhurst AD (2003) Differences in the central nervous system distribution and pharmacology of the mouse 5-hydroxytryptamine-6 receptor compared with rat and human receptors investigated by radioligand binding, site-directed mutagenesis, and molecular modeling. *Mol Pharmacol* **64:**1295–1309.

Kohen R, Fashingbauer LA, Heidmann DE, Guthrie CR, and Hamblin MW (2001) Cloning of the mouse 5-HT6 serotonin receptor and mutagenesis studies of the third cytoplasmic loop. *Brain Res Mol Brain Res* **90:**110–117.

Kohen R, Metcalf MA, Khan N, Druck T, Huebner K, Lachowicz JE, Meltzer HY, Sibley DR, Roth BL, and Hamblin MW (1996) Cloning, characterization and chromosomal localization of a human 5- HT6 serotonin receptor. *J Neurochem* **66:**47–56.

Kroeze WK, Kristiansen K, and Roth BL (2002) Molecular biology of serotonin receptors: structure and function at the molecular level. *Curr Top Med Chem* **2:**507–528.

Monsma FJ Jr, Shen Y, Ward RP, Hamblin MW, and Sibley DR (1993) Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. *Mol Pharmacol* **43:**320–327.

Rogers DC and Hagan JJ (2001) 5-HT6 receptor antagonists enhance retention of a water maze task in the rat. *Psychopharmacology (Berl)* **158:**114–119.

Roth BL, Craigo SC, Choudhary MS, Uluer A, Monsma FJ Jr, Shen Y, Meltzer HY, and Sibley DR (1994) Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. *J Pharmacol Exp Ther* **268:**1403–1410.

Ruat M, Traiffort E, Arrang JM, Tardivel-Lacombe J, Diaz J, Leurs R, and Schwartz JC (1993) A novel rat serotonin (5-HT6) receptor: molecular cloning, localization and stimulation of cAMP accumulation. *Biochem Biophys Res Commun* **193:**268–276.

Tsai SJ, Liu HC, Liu TY, Wang YC, and Hong CJ (1999) Association analysis of the 5-HT6 receptor polymorphism C267T in Alzheimer's disease. *Neurosci Lett* **276:**138–139.

Ward RP, Hamblin MW, Lachowicz JE, Hoffman BJ, Sibley DR, and Dorsa DM (1995) Localization of serotonin subtype 6 receptor messenger RNA in the rat brain by in situ hybridization histochemistry. *Neuroscience* **64:**1105–1111.

Woolley ML, Bentley JC, Sleight AJ, Marsden CA, and Fone KC (2001) A role for 5-ht6 receptors in retention of spatial learning in the Morris water maze. *Neuropharmacology* **41:**210–219.

Address correspondence to: Dr. Bryan Roth, Department of Biochemistry, Psychiatry, and Neurosciences, Case Western University, 10900 Euclid Avenue, Room RT-500, Cleveland, OH 44106-4936. E-mail: bryan.roth@case.edu