International Journal of Neuropsychopharmacology (1999), **2**, 145–150.

Knockout Corner
5-HT$_{1B}$ receptor knockout mice: a review

Kimberly L. Stark and René Hen

Columbia University, Center for Neurobiology and Behavior, 722 West 168th Street, New York, New York 10032

Abstract

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter involved in a number of physiological functions including sleep, appetite, pain perception, and sexual activity. Several pathological states such as migraine, depression, and anxiety have been linked to the serotonergic system, and serotonergic drugs have been used to treat these disorders. To date, there are 14 known serotonin receptor subtypes through which serotonin exerts its multiple actions. The classic pharmacological approach to study how these individual receptor subtypes contribute to various behaviours has been to use selective drugs that either block or activate certain receptor subtypes, and then study the effects of these compounds on physiology and behaviour. A complementary genetic approach is the technique of gene targeting. Using this technology, we and others have begun to examine the contribution of several serotonin receptor subtypes to complex behaviours through the generation of knockout mice that lack the genes encoding these receptors. In this review, we will describe what we have learned about the serotonergic system and the function of the 5-HT$_{1B}$ receptor by the analysis of 5-HT$_{1B}$ receptor knockout mice. Furthermore, we will discuss the implications of these findings and our plans for future studies.

Received 9 December 1998; Reviewed 22 February 1999; Revised 21 March 1999; Accepted 22 March 1999

Key words: Serotonin, knockout, animal model, genetic disease, psychiatric disorder.

Introduction

The 5-HT$_{1B}$ receptor is expressed predominantly in the basal ganglia and to a lesser extent in other regions including the hippocampus, central grey, and raphé nuclei. It is both an autoreceptor, localized presynaptically on serotonergic terminals where it inhibits the release of serotonin, and a heteroreceptor, located on other nerve endings where it regulates the release of various neurotransmitters (Boschert et al., 1994, Ghavami et al., 1999). Pharmacological studies have indicated that activation of 5-HT$_{1B}$ receptors may lead to an increase in anxiety and locomotion, and a decrease in food intake, sexual activity, and aggressive behaviour (Briley et al., 1997).

In order to examine the contribution of this receptor subtype to these and other behaviours, homozygous mutant mice lacking the 5-HT$_{1B}$ receptor were created (Saudou et al., 1994). This was achieved by homologous recombination, replacing part of the 5-HT$_{1B}$ coding sequence with a neomycin phosphotransferase gene (*neo*). The resulting mutant animals were on a pure 129/Sv genetic background. The initial analysis of these mutants revealed that they did not display any obvious developmental, anatomical, or behavioural abnormalities. They were fertile, and heterozygote crossings led to the expected Mendelian ratios.

Address for correspondence: Dr René Hen, Columbia University, Center for Neurobiology and Behavior, 722 West 168th Street, New York, New York 10032.
Tel.: (212) 543-5328 *Fax*: (212) 543-5410.
E-mail: rh95@columbia.edu

Evaluation of 5-HT$_{1B}$ receptor knockout mice

Many behavioural and physiological differences found between 5-HT$_{1B}$ receptor knockout mice and wild-type mice have been discovered, both with and without pharmacological challenge. For example, a role for the 5-HT$_{1B}$ receptor in regulating the effects of fenfluramine, a widely prescribed appetite suppressant, has recently been found by using knockout mice (Lucas et al., 1998). By using a food intake behavioural paradigm, it was found that fenfluramine's hypophagic effect was absent in 5-HT$_{1B}$ knockout mice, indicating that stimulation of the 5-HT$_{1B}$ receptor was necessary for this response. An involvement of the 5-HT$_{1B}$ receptor in mediating the effects of MDMA (or ecstasy), has also been found by using these mice (Scearce-Levie et al., 1999). In this case, knockout mice displayed a reduced locomotor response to

Table 1. Phenotypes of the 5-HT_{1B} knockout mouse*/comparisons to agonist and antagonist effects†

Treatment or measurement	5-HT_{1B} KO phenotype	Effect of 5-HT_{1B} agonists‡ (Agonist/effect)	Effect of 5-HT_{1B} antagonists‡ (Antagonist/effect)
5-HT	= Total tissue content[23]		
	= In vivo microdialysis[2]	Sumatriptan/↓(FC)[2]	
	(FC, VH)	CP93129/↓(FC, VH)[2]	GR127935/=[24]
	Electrically-evoked release[3]	Sumatriptan/↓(H, FC)[3]	
	= (FC)/↑(M, H)	CP93129/↓(H, FC)[3]	
Body weight	=[4]/↑[6, 7]		
Feeding	=[4, 5, 17]	RU24969/↓[4]	
Exploratory activity	↑ Open-field[20]		
	↑ Novel object exploration[9]		
General activity	↑ 24 h monitoring[6]/↑§[6]	RU24969/↑[1]	
Anxiety	= Light/dark choice test[8]		
	↓ Open-field (↑ in path centre/total path)[20]		
	↓ Ultrasound vocalizations§[6]		
	=[6, 9]/↓[6] Elevated plus maze		
	↓ Burying behaviour[19]		
Startle responses	↓ Startle reactivity[10]	RU24969/↓[10]	
	↑ Prepulse inhibition[10]	RU24969/↓[10]	GR127935/↑[10]
	= Habituation[10]	RU24969/↓[10]	
Aggressive behaviour	↑ Resident–intruder test (males)[1]	Eltoprazine/↓[11]	GR127935/=[11]
	↑ Maternal aggression (females)[11]		
Sleep	↑ Paradoxical sleep	CP94253/↓[12]	
	(light phase)[12]	RU24969/↓[12]	GR127935/↑[12]
	↓ Slow wave sleep (light phase)[12]		
Cocaine	↑ Locomotor responses[17]		GR127935/↓[26]
	↓ Stereotopies[17]		
	Self-administration		
	↓ Latency to meet IV self-administration criteria[16]		
	= Fixed ratio schedule[17]		
	↑ Progressive ratio schedule[17]	RU24969/↑[22]‖, CP94253/↑[22]‖	GR127935/=[25, 22]‖
	↓ c-fos induction (Str)[18]	RU24969/↑(Str)[18]	GR127935/↓(Str)[18]
	↓ Conditioned place preference[21]		
Ethanol	↑ Self-administration[5]		
	↓ Ethanol-induced ataxia[5]		
	= Ethanol metabolism[5]		
	= Ethanol withdrawal reactions[5]		
	= Conditioned taste aversion[14]		
	↓ Conditioned place preference[14]		
Fenfluramine	↓ Anorectic effect[4]	RU24969/↑[4]	
	↓ c-fos induction (PVN, CeA, BNST)[4]	RU24969/↑ (CeA, BNST)[4]	
MDMA	↓ Open-field locomotion[15]		GR127935/↓[15]
Morphine	↑ Sensitivity to the analgesic effects of morphine[13] (tail immersion test)		

= No difference compared to wild-type mice.
↑ Increased compared to wild-type mice.
↓ Decreased compared to wild-type mice.
* Adult phenotypes, unless otherwise noted.
† In our 129/Sv strain of mice, unless otherwise noted.
‡ In wild-type mice.
§ As observed in pups.
‖ As observed in rats.

M, Midbrain
FC, Frontal cortex
VH, Ventral hippocampus
PVN, Paraventricular nucleus of the hypothalamus
CeA, Central amygdaloid nucleus
BNST, Bed nucleus of the stria terminalis
Str, Striatum
H, Hippocampus

this popular psychoactive, drug of abuse. A role for the 5-HT$_{1B}$ receptor in modulating the effects of SSRIs (selective serotonin reuptake inhibitors) has also been suggested. In microdialysis studies, fluoxetine and paroxetine administration resulted in larger increases in extracellular serotonin levels in knockout than in wild-type mice (Trillat et al., unpublished observations).

What is perhaps most striking, and seems to have a common theme, is that these 5-HT$_{1B}$ receptor knockout mice have been found to be vulnerable to drugs of abuse. This has been found in both cocaine (Rocha et al., 1998a) and alcohol self-administration studies (Crabbe et al., 1996). Interestingly, impulsiveness and aggressiveness, two traits often associated with drug abuse, have also been found to be increased in knockout mice (Brunner and Hen, 1997; Ramboz et al., 1996; Saudou et al., 1994).

Cocaine has been found to act on the dopamine, norepinephrine, and serotonin transporters. Although this drug has a higher affinity for the serotonin, rather than the dopamine transporter (Ritz and Kuhar, 1989), much attention has been focused on the dopamine system. However, several studies have proposed that the serotonergic system may play a role in the rewarding properties of cocaine (Parsons et al., 1998; Rocha et al., 1998b). The 5-HT$_{1B}$ receptor knockout mouse has provided one avenue to explore whether this receptor contributes to the effects of cocaine. When given the opportunity to self-administer cocaine on a progressive ratio schedule, knockout mice were found to work harder to obtain cocaine than their wild-type counterparts. Knockout mice would press up to 25 times to obtain a dose of cocaine whereas wild-type mice would press only 9 times (Rocha et al., 1998a). This effect was specific for cocaine; when cocaine was replaced by saline, this effect was eliminated. Further, this behaviour was not found with all types of rewards. Food intake, for example, was the same between groups.

Similarly, when given free access to either an ethanol-containing solution or water, knockout mice were found to drink twice as much ethanol as wild-type mice (Crabbe et al., 1996). Again, this effect was found to be drug specific; substitutions of sucrose, saccharin, or quinine produced no difference in intake between the groups. The observations with cocaine and alcohol self-administration have thus led us to believe that the 5-HT$_{1B}$ receptor knockout mouse may represent a genetic model of vulnerability to drugs of abuse.

As a classic knockout, these 5-HT$_{1B}$ receptor knockout mice have developed throughout their lifetime with an absence of this receptor. As such, compensations and changes in other neurotransmitter systems may have occurred. In fact, we have behavioural and biochemical evidence that such changes have taken place. A first indication that compensations had occurred was that administration of the 5-HT$_{1B/1D}$ receptor antagonist GR127935 failed to mimic an enhanced locomotor response to cocaine, which had been found in knockout mice. Further evidence of compensatory changes is that drugs acting specifically on the DA system have been found to cause profound behavioural differences in 5-HT$_{1B}$ receptor knockout mice, compared to wild-type mice (Scearce et al., 1997). GBR12909, a specific DA uptake blocker, was found to increase locomotion in both genotypes, but by three times more in knockout mice. Additionally, SKF81297, a D1 receptor agonist, was found to increase locomotion in both genotypes, but significantly more in knockout mice during the first 20 min post-injection. Further experiments, more directly addressing whether changes have taken place in the DA system, found increases in D1 dopamine receptor expression in the striatum.

Interestingly, it has been found that drug-naive 5-HT$_{1B}$ knockout mice are in a behavioural and biochemical state, resembling that of wild-type mice which have undergone chronic drug treatments. In striatal samples, 5-HT$_{1B}$ receptor knockout mice were found to possess a higher basal level of both ΔFosB, a splice variant of the transcription factor FosB, and of the AP-1 transcriptional complex (Rocha et al., 1998a). The AP-1 transcriptional complex is composed of heterodimers formed by members of the Fos and Jun families of transcription factors. This complex binds to a DNA motif found in several

References to Table 1

[1] Saudou et al. (1994)
[2] Trillat et al. (1997)
[3] Pineyro et al. (1995)
[4] Lucas et al. (1998)
[5] Crabbe et al. (1996)
[6] Brunner et al. (In Press)
[7] Olivier et al. (unpublished)
[8] Ramboz et al. (1996)
[9] Malleret et al. (unpublished)
[10] Dulawa et al. (1997)
[11] Ramboz et al. (unpublished observations)
[12] Boutrel et al. (1999)
[13] Hain et al. (unpublished observations)
[14] Risinger et al. (1996)
[15] Scearce-Levie et al. (1999)
[16] Rocha et al. (1997)
[17] Rocha et al. (1998a)
[18] Lucas et al. (1997)
[19] C. Lopez-Rubalcava (unpublished)
[20] Zhuang et al. (In Press)
[21] Scearce-Levie and Belzung (unpublished)
[22] Parsons et al. (1998)
[23] M. Hamon (unpublished observations)
[24] Gardier et al. (unpublished observations)
[25] Rocha et al. (unpublished observations)
[26] Scearce et al. (1997)

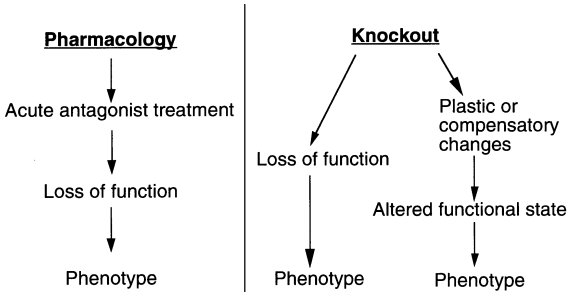

Figure 1. Comparison of phenotypes produced by antagonists and gene knockouts. Acute antagonist treatment may lead to particular phenotype through a loss of function. A gene knockout may produce a similar phenotype, or one which results from compensatory changes which have taken place during development.

promoter regions and thus regulates the transcription of downstream genes. Chronic drug treatments including cocaine (Rocha et al., 1998a), morphine (Nye and Nestler, 1995), methamphetamine (Ishihara et al., 1996), and ethanol (Wang et al., 1998) have been shown to produce increases in ΔFosB and/or the AP-1 complex. We have, therefore, hypothesized that the altered state found in knockout mice may be responsible for their increased vulnerability to cocaine and other drugs of abuse such as ethanol. Based upon the localization of the 5-HT_{1B} receptor in the dopaminergic reward pathway, we have also hypothesized that there may be increased dopaminergic transmission, since the 5-HT_{1B} receptor is no longer present on GABAergic terminals projecting to the substantia nigra and ventral tegmental area. This may result in heightened responsiveness to drugs of abuse.

Pharmacological tool or genetic model?

It is clear from the examples cited above, and those shown in Table 1, that in some cases 5-HT_{1B} knockout mice can be used as a pharmacological tool. In particular, these mice can be used to assess whether specific drugs require the 5-HT_{1B} receptor to be effective. We have shown, for example, that the effects of sumatriptan on dural inflammation are mediated by 5-HT_{1B} receptors in mice (Yu et al., 1996). Similarly, we have shown that the anorectic effect of the 5-HT releaser fenfluramine, as well as the effect of fenfluramine on c-fos expression in the paraventricular nucleus of the hypothalamus, requires activation of the 5-HT_{1B} receptor (Lucas et al., 1998).

However, in several instances, we have also shown that the effect of 5-HT_{1B} antagonists are distinct from those of knockout mice. For example, the acute administration of the 5-$HT_{1B/1D}$ antagonist GR127935 decreases the locomotor effect of cocaine and has no effect on cocaine self-administration in wild-type mice (Scearce et al., 1997; Rocha, unpublished observations), while in 5-HT_{1B} knockout mice, both the locomotor and rewarding effects of cocaine are enhanced (Rocha et al., 1998a). Such discrepancies are likely to result from compensatory changes that took place during the development of knockout mice. Thus, these mice may better serve as models of genetic disorders or chronic drug treatments, than tools to study the consequence of the acute blockade of a receptor (see Figure 1).

We are currently exploring the usefulness of the 5-HT_{1B} knockout as a genetic model. A quantitative trait locus (QTL) associated with differential responsiveness to drugs of abuse has been mapped in the vicinity of the 5-HT_{1B} gene (Crabbe et al., 1994). Analysis of the gene encoding the 5-HT_{1B} receptor in various inbred strains, as well as in the human population, may lead to some insights into genetic vulnerability to drugs of abuse, as well as into other genetic susceptibilities (Grailhe et al., unpublished observations). One such study, conducted in two human populations, found polymorphisms in the 5-HT_{1B} gene to be associated with antisocial personality disorder and alcoholism (Lappalainen et al., 1998). It is therefore possible that the 5-HT_{1B} gene is one of the many genetic determinants that may underlie vulnerability to drugs of abuse and other genetic disorders.

Conclusions

As we have seen, the study of the 5-HT_{1B} receptor knockout mouse has led to a number of interesting observations. These studies have demonstrated a role for this receptor in modulating the effects of popular drugs such as fenfluramine, ethanol, MDMA, and cocaine. Additionally, we have begun to learn about the complicated interplay between the dopaminergic and serotonergic neurotransmitter systems. With the creation of the 5-HT_{1B} knockout mouse, it appears that we have created a genetic model of vulnerability to drugs of abuse from which we still have much to learn. Future studies will be directed at unravelling the circuitry underlying many of these behaviours by the creation of tissue-specific knockout and rescue mice, and through inducible strategies aimed to further study the function of the 5-HT_{1B} receptor, without the complications of developmental compensations (see Stark et al., 1998). Other avenues to be pursued will include studies of the interaction of the 5-HT_{1B} receptor with other moelcules (such as ΔFosB) by the generation of double-knockout mice, and the evaluation of the molecular changes or adaptations that may be responsible for the knockout phenotype.

References

Boschert U, Aït Amara D, Segu L, Hen R (1994). The mouse 5-hydroxytryptamine$_{1B}$ receptor is localized predominantly on axon terminals. *Neuroscience 58*, 167–182.

Boutrel B, Franc B, Hen R, Hamon M, Adrien J (1999). Key role of 5-HT$_{1B}$ receptors in the regulation of paradoxical sleep as evidenced in 5-HT$_{1B}$ knock-out mice. *Journal of Neuroscience 19*, 3204–3212.

Briley M, Chopin P, Marien M, Moret C (1997). Functional neuropharmacology of compounds acting at 5-HT1$_{B/D}$ receptors. In: Baumgarten HG, Göthert M (Eds.), *Serotoninergic Neurons and 5-HT Receptors in the CNS* (pp. 269–291). Berlin: Springer-Verlag.

Brunner D, Buhot MC, Hen R, Hofer M (In Press). Anxiety, motor activation, and maternal–infant interactions in 5-HT$_{1B}$ knockout mice. *Behavioural Neuroscience*.

Brunner D, Hen R (1997). Insights into the neurobiology of impulsive behavior from serotonin receptor knockout mice. In: Stoff DM, Mann JJ (Eds.), *The Neurobiology of Suicide: from the Bench to the Clinic* (pp. 81–105). New York: New York Academy of Science.

Crabbe JC, Belknap JK, Buck KJ (1994). Genetic animal models of alcohol and drug abuse. *Science 264*, 1715–1723.

Crabbe JC, Phillips TJ, Feller DJ, Hen R, Wenger CD, Lessov CN, Schafer GL (1996). Elevated alcohol consumption in null mutant mice lacking 5-HT$_{1B}$ receptors. *Nature Genetics 14*, 98–101.

Dulawa SC, Hen R, Scearce-Levie K, Geyer MA (1997). Serotonin$_{1B}$ receptor modulation of startle reactivity, habituation, and pre-pulse inhibition in wild-type and serotonin$_{1B}$ knockout mice. *Psychopharmacology 132*, 125–134.

Ghavami A, Stark KL, Jareb M, Ségu L, Hen R (1999). Differential addressing of 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors in epithelial cells and neurons. *Journal of Cell Science 112*, 967–976.

Ishihara T, Akiyama K, Kashihara K, Ujike H, Hamamura T, Okada S, Kuroda S (1996). Activator protein-1 binding activities in discrete regions of rat brain after acute and chronic administration of methamphetamine. *Journal of Neurochemistry 67*, 708–716.

Lappalainen J, Long JC, Eggert M, Ozaki N, Robin R, Brown GL, Naukkarinen H, Virkkunen M, Linnoila M, Goldman D (1998). Linkage of antisocial alcoholism to the serotonin 5-HT1B receptor gene in 2 populations. *Archives of General Psychiatry 55*, 989–994.

Lucas JJ, Segu L, Hen R (1997). 5-hydroxyytryptamine$_{1B}$ receptors modulate the effect of cocaine on c-fos expression: converging evidence using 5-hydroxytryptamine$_{1B}$ knockout mice and the 5-hydroxytryptamine$_{1B/1D}$ antagonist GR127935. *Molecular Pharmacology 51*, 755–763.

Lucas JJ, Yamamoto A, Scearce-Levie K, Saudou F, Hen R (1998). Absence of fenfluramine-induced anorexia and reduced c-fos induction in the hypothalamus and central amygdaloid complex of serotonin 1B receptor knock-out mice. *Journal of Neuroscience 18*, 5537–5544.

Nye HE, Nestler EJ (1995). Induction of chronic fos-related antigens in rat brain by chronic morphine administration. *Molecular Pharmacology 49*, 636–645.

Parsons LH, Weiss F, Koob GF (1998). Serotonin$_{1B}$ receptor stimulation enhances cocaine reinforcement. *Journal of Neuroscience 18*, 10078–10089.

Pineyro G, Castanon N, Hen R, Blier P (1995). Regulation of [^{3}H]5-HT release in raphe, frontal cortex and hippocampus of 5-HT-1B knockout mice. *NeuroReport 7*, 353–359.

Ramboz S, Saudou F, Aït Amara D, Belzung C, Segu L, Misslin R, Buhot MC, Hen R (1996). 5-HT$_{1B}$ receptor knockout – behavioral consequences. *Behavioral Brain Research 73*, 305–312.

Risinger FO, Bormann NM, Oakes RA (1996). Reduced sensitivity to ethanol reward, but not ethanol aversion, in mice lacking 5-HT$_{1B}$ receptors. *Alcoholism: Clinical and Experimental Research 20*, 1401–1405.

Ritz MC, Kuhar MJ (1989). Relationship between self-administration of amphetamine and monoamine receptors in brain: comparison with cocaine. *Journal of Pharmacology and Experimental Therapeutics 248*, 1010–1016.

Rocha BA, Ator R, Emmett-Oglesby MW, Hen R (1997). Intravenous cocaine self-administration in mice lacking 5-HT$_{1B}$ receptors. *Pharmacology, Biochemistry and Behaviour 57*, 407–412.

Rocha BA, Fumagalli F, Gainetdinov RR, Jones SR, Ator R, Giros B, Miller GW, Caron MG (1998b). Cocaine self-administration in dopamine-transporter knockout mice. *Nature Neuroscience 1*, 132–137.

Rocha BA, Scearce-Levie K, Lucas JJ, Hiroi N, Castanon N, Crabbe JC, Nestler EJ, Hen R (1998a). Increased vulnerability to cocaine in mice lacking the serotonin-1B receptor. *Nature 393*, 175–178.

Saudou F, Aït Amara D, Dierich A, LeMeur M, Ramboz S, Segu L, Buhot MC, Hen R (1994). Enhanced aggressive behavior in mice lacking 5-HT$_{1B}$ receptors. *Science 265*, 1875–1878.

Scearce K, Lucas J, Castanon N, Segu L, Arango V, Hen R (1997). Dopaminergic compensations in knockout mice lacking the serotonin 1B receptor. *Society for Neuroscience Abstracts 23*, 518.

Scearce-Levie K, Viswanathan SS, Hen R (1999). Locomotor response to MDMA is attenuated in knockout mice lacking the 5-HT$_{1B}$ receptor. *Psychopharmacology 141*, 154–161.

Stark KL, Oosting RS, Hen R (1998). Novel strategies to probe the functions of serotonin receptors. *Biological Psychiatry 44*, 163–168.

Trillat AC, Malagié I, Scearce K, Pons D, Anmella MC, Jacquot C, Hen R, Gardier AM (1997). Regulation of serotonin release in the frontal cortex and ventral hippocampus of homozygous mice lacking 5-HT$_{1B}$ receptors: in vivo microdialysis studies. *Journal of Neurochemistry 69*, 2019–2025.

Wang XD, Liu C, Chung J, Stickel F, Seitz HK, Russell RM

(1998). Chronic alcohol intake reduces retinoic acid concentration and enhances AP-1 (c-Jun and c-Fos) expression in rat liver. *Hepatology 28*, 744–750.

Yu XJ, Waeber C, Castanon N, Scearce K, Hen R, Macor JE, Chaveau J, Moskovitz A (1996). 5-Carboxamidotryptamine, CP-122,288 and dihydroergotamine but not sumatriptan, CP-93129, and serotonin-5-*O*-carboxymethyl-glycyl-tyrosinamide block dural plasma protein extravasation in knockout mice that lack 5-hydroxytryptamine$_{1B}$ receptors. *Molecular Pharmacology 49*, 761–765.

Zhuang X, Gross C, Santarelli L, Compan V, Hen R (In Press). Altered emotional states in knockout mice lacking the 5-HT-1A or 5-HT-1B receptors. *Neuropsychopharmacology*.