Knockout Corner: 5-HT$_{1A}$ receptor inactivation: anxiety or depression as a murine experience

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Abstract
5-HT$_{1A}$ receptors play a critical role in the pathophysiology of anxiety and depression as well as in the mode of action of anxiolytic and antidepressant drugs. Mice with a targeted inactivation of the 5HT$_{1A}$ receptor show a phenotype that is associated with a gender-modulated and gene/dose-dependent increase of anxiety-related and antidepressant-like behaviours. Since this behavioural phenotype was observed in animals in which the mutation was bred into mice of different genetic backgrounds, 5-HT$_{1A}$ receptor knockout mice represent a useful model system for advanced investigations of 5-HT$_{1A}$ genotype/phenotype interaction.

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Introduction
A possible role for the 5-HT$_{1A}$ receptor in the modulation of anxiety and depression as well as in the mode of action of anxiolytic and antidepressant drugs has been suspected for many years. 5-HT$_{1A}$ receptors operate both as somatodendritic autoreceptors and as postsynaptic receptors. Somatodendritic 5-HT$_{1A}$ autoreceptors are predominantly located on 5-HT neurons and dendrites in the midbrain raphe complex and their activation by 5-HT or 5-HT$_{1A}$ agonists decreases the firing rate of serotonergic neurons and subsequently reduces the release of 5-HT from nerve terminals (Figure 1) (Hamon et al., 1988; Sprouse and Aghajanian, 1986).

Postsynaptic 5-HT$_{1A}$ receptors are widely distributed in forebrain regions that receive serotonergic input, notably in the cortex, hippocampus and hypothalamus. Their activation results in neuronal inhibition, the consequences of which are not well understood, and in physiological responses that depend upon the function of the target cells (e.g. activation of the hypothalamic–pituitary–adrenocortical system) (Hamon et al., 1990).

5-HT$_{1A}$ receptor expression is modulated by steroid hormones and 5-HT$_{1A}$-mediated signalling is an important regulator of gene expression through its coupling to G-proteins that inhibit adenylyl cyclase.

At the end of 1998 a series of three papers reported the generation of mice with a targeted inactivation of the 5-HT$_{1A}$ receptor (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998). Functional 5-HT$_{1A}$ receptor knockout was confirmed by a complete lack of [3H]8-OH-DPAT or [125I]MPPI binding to brain 5-HT$_{1A}$ receptors in null-mutant (−/−) mice, with intermediate binding in heterozygote (+/−) mice. Intriguingly, all three knockout mouse strains display a similar behavioural phenotype characterized by increased anxiety-related and antidepressant-like behaviour in several different paradigms.

This review focuses on the behavioural and physiological consequences of a constitutive knockout (KO) of the 5-HT$_{1A}$ receptor gene in mice and emphasizes its implications for the 5-HT hypothesis of anxiety disorders and depression as well as for novel anxiolytic and antidepressant drug development.

Anxiety-related behaviour
Mice with a targeted inactivation of the 5-HT$_{1A}$ receptor consistently display a spontaneous phenotype that is associated with a gender-modulated and gene/dose-dependent increase of anxiety-related behaviours (Table 1) (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998). With the exception of an enhanced sensitivity of terminal 5-HT$_{1B}$ receptors, no major neuroadaptational
Figure 1. Hypothetical mechanism of physiological and behavioural consequences of targeted inactivation of the 5HT₁A receptor gene in mice. Anxiety-related and antidepressant-like behaviour in 5-HT₁A receptor knockout mice may represent a consequence of increased terminal 5-HT availability resulting from the lack or reduction in presynaptic somatodendritic 5-HT₁A autoreceptor negative feedback function. Indirect evidence for increased presynaptic serotoninergic activity is provided by the compensatory up-regulation of terminal 5-HT₁B receptors. While increased 5-HT availability and activation of other serotoninergic receptor subtypes that have been shown to mediate anxiety (e.g. 5-HT₂C receptor) may contribute to increased anxiety in rodent model, multiple downstream neurotransmitter pathways or neurocircuits, including noradrenergic, GABAergic, glutamatergic, and peptidergic transmission are likely to participate in the processing of this complex behavioural trait.

Table 1. Behavioural phenotype of 5-HT₁A knockout mice

<table>
<thead>
<tr>
<th>Genetic background</th>
<th>Anxiety-related behaviour</th>
<th>Antidepressant-like behaviour</th>
<th>Locomotor activity</th>
<th>Motor coordination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parks et al. (1998)</td>
<td>129Sv into Swiss–Webster</td>
<td>OF: ↑↑, m, ↑↑↑</td>
<td>↑↑↑</td>
<td>↑f</td>
</tr>
<tr>
<td>Ramboz et al. (1998)</td>
<td>129Sv</td>
<td>OF: ↑↑, m, ↑↑↑</td>
<td>↑↑↑</td>
<td>↑</td>
</tr>
<tr>
<td>Heisler et al. (1998)</td>
<td>129Sv into C57BL/6J</td>
<td>OF: ↑ (+/+ &lt; −/−)</td>
<td>↑</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EPM: ↑↑↑</td>
<td>↑↑↑ (+/− &lt; −/−)</td>
<td>↑↑↑ (+/− &lt; −/−)</td>
</tr>
</tbody>
</table>

* OF, open field; EPM, elevated plus-maze; EZM, elevated zero-maze; NO, novel objects.
† FS, forced swim; TS, tail suspension.
‡ Rotorod.
f, Female; m, male; −, no change; nd, not done; +/− < −/−, gene/dose-effect.

Changes were detected (Table 2). It is worth noting that this behavioural phenotype was observed in animals in which the mutation was bred into mice of Swiss–Webster, C57BL/6J and 129/Sv backgrounds, solidly substantiating the assumption that this behaviour is an authentic consequence of reduced or absent 5-HT₁A receptors. While all research groups used open-field exploratory behaviour as a model for assessing anxiety, two groups confirmed that 5-HT₁A knockout mice had increased anxiety by using other models, the elevated zero-maze or elevated plus-maze test (Heisler et al., 1998; Ramboz et al., 1998). These ethologically based conflict models test fear and anxiety-related behaviours based on the natural tendencies for rodents to prefer enclosed, dark spaces versus their interest in exploring novel environments.

Activation of presynaptic 5-HT₁A receptors provide the brain with an autoinhibitory feedback system controlling 5-HT neurotransmission (Figure 1). Thus, en-
hanced anxiety-related behaviour most likely represents a consequence of increased terminal 5-HT availability resulting from the lack or reduction in presynaptic somatodendritic 5-HT$_{1A}$ autoreceptor negative feedback function. Although brain tissue 5-HT and levels and 5-HT turnover of were unchanged in 5-HT$_{1A}$ KO mice, indirect evidence for increased presynaptic serotonergic activity resulting in elevated synaptic 5-HT concentrations is provided by the compensatory up-regulation of terminal, 5-HT release inhibiting 5-HT$_{1B}$ receptors. This mechanism is also consistent with recent theoretical models of fear and anxiety that are primarily based upon pharmacologically derived data. The cumulative reduction in serotonergic impulse flow to septohippocampal and other limbic and cortical areas involved in the control of anxiety is believed to explain the anxiolytic effects of ligands with selective affinity for the 5-HT$_{1A}$ receptor in some animal models of anxiety-related behaviour. This notion is based, in part, on evidence that 5-HT$_{1A}$ agonists (e.g. 8-OH-DPAT) and antagonists (e.g. WAY-100635) have anxiolytic or anxiogenic effects, respectively. However, to complicate matters further, 8-OH-DPAT has anxiolytic effects when injected in the raphe nucleus, whereas it is anxiogenic when applied to the hippocampus. Thus, stimulation of postsynaptic 5-HT$_{1A}$ receptors has been proposed to elicit anxiogenic effects, while activation of 5-HT$_{1A}$ autoreceptors is thought to induce anxiolytic effects via suppression of serotonergic neuronal firing resulting in attenuated 5-HT release in limbic terminal fields.

Although there is converging evidence that the 5-HT$_{1A}$ receptor mediates anxiety-related behaviour, the precise mechanism that renders 5-HT$_{1A}$ receptor-deficient mice more anxious remains to be elucidated. While increased 5-HT availability and activation of other serotonergic receptor subtypes that have been shown to mediate anxiety (e.g. 5-HT$_{2A}$ receptor) may contribute to increased anxiety in rodent models, multiple downstream neurotransmitter pathways or neurocircuits, including noradrenergic, GABAergic, glutamatergic, and peptidergic transmission, as suggested by overexpression or targeted inactivation of critical genes within these systems (Kash et al., 1999; Palmier et al., 1992; Sallinen et al., 1998; Timpl et al., 1998), have been implicated to participate in the processing of this complex behavioural trait (Figure 1).

### Table 2. Neuroadaptive changes in 5-HT$_{1A}$ knockout mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>5-HT$_{1A}$ Receptor Binding$^{1,3}$</th>
<th>WAY-100635 8-OH-DPAT$^2$</th>
<th>5-HT$_{1B}$ Hypothermia$^3$</th>
<th>5-HT$<em>{1A}$/5-HT$</em>{1B}$ ratio</th>
<th>5-HT$_{1B}$ 5-HT$^5$</th>
<th>TH$^0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ / −</td>
<td>50–65% (~ 50%) nd nd 80–90% nd</td>
<td>− − −</td>
<td>− − −</td>
<td>− − −</td>
<td>− − −</td>
<td>− − −</td>
</tr>
<tr>
<td>− / −</td>
<td>0 0 0 0 0 0</td>
<td>†</td>
<td>− − −</td>
<td>− − −</td>
<td>− − −</td>
<td>− − −</td>
</tr>
</tbody>
</table>

1 Parks et al. (1998); 2 Ramboz et al. (1998); 3 Heisler et al. (1998).
−, No change; 0, absent; nd, not done

Antidepressant-like behaviour

5-HT$_{1A}$ receptor KO mice also show genotype-dependent and background strain-unrelated antidepressant-like behaviour in two models of behavioural despair, the forced-swim and tail suspension tests (Table 1) (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998). The reduced immobility in antidepressant/stress test models is probably also due to an increased serotonergic tone resulting from the compromised 5-HT$_{1A}$ autoreceptor-dependent negative feedback regulation.

Among the fourteen 5-HT receptor subtypes now identified, the role of 5-HT$_{1A}$ receptors in the therapeutic action of antidepressant drugs is attracting extraordinary interest; however, there is substantial conflicting evidence regarding involvement of other serotonergic receptor subtypes and neurotransmitter systems or neurocircuits that interact with 5-HT neurotransmission. While the exact neuroadaptive mechanism of antidepressant action of selective 5-HT reuptake inhibitors (SSRIs) remains largely unknown, as the onset of clinical improvement frequently takes 2–3 wk or more after initiation of SSRIs administration, progressive functional desensitization of pre- and postsynaptic serotonergic receptors, including the 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{2A}$ that are initiated by blockade of the 5-HT transporter, have been implicated in these delayed therapeutic effects.

Electrophysiological studies in rats indicate that each class of antidepressants enhance 5-HT neurotransmission via differential adaptive changes in the 5-HT$_{1A}$ receptor-modulated negative feedback regulation that eventually
leads to an overall increase of terminal 5-HT (Blier and de Montigny, 1998). Desensitization of 5-HT$_{1A}$ receptors has been reported in rodents (Le Poul et al., 1995; Li et al., 1993) and humans (Berlin et al., 1998; Lerer et al., 1999; Lesch et al., 1991; Sargent et al., 1997). Since no changes in the density of 5-HT$_{1A}$ receptor (and other 5-HT receptor subtypes) were detected, desensitization is unlikely to result from downregulation of these receptors (Le Poul et al., In Press; Li et al., 1997). The dissociation between receptor number and functional responsiveness has led to the investigation of possible post-receptor sites of actions of these drugs and there is a growing body of evidence that the action of antidepressants involves direct or indirect modification of signal transduction as an integral part of neuroadaptational mechanisms on which antidepressant drug efficacy is based.

Mice with a disrupted 5-HT transporter gene have been suggested as an alternative model to pharmacological studies of SSRI-evoked antidepressant mechanisms to assess the hypothesized association between 5-HT uptake function and 5-HT$_{1A}$ receptor desensitization (Bengel et al., 1998; Murphy et al., 1999). Excess serotonergic neurotransmission in mice lacking 5-HT transport results in desensitized and, unlike observations following SSRI administration, downregulated 5-HT$_{1A}$ receptors in the midbrain raphe complex but not in the hippocampus (Fabre et al., In Press; Li et al., 1999) and is suspected to play a role in the increased anxiety-related and antidepressant-like behaviours in these mice using the light–dark box, elevated zero-maze, tail suspension paradigms (Murphy DL, personal communication: May 1999). In contrast to 5-HT$_{1A}$ KO mice anxiety-related behaviour, which can be reversed by anxiolytics of the benzodiazepine type, is more pronounced in female 5-HT transporter −/− mice.

Clinical implications

There is a growing body of evidence that 5-HT may modulate anxiety and depression in a reciprocal mode, with high serotonergic activity being associated with anxiety and low activity with depression. However, the finding that 5-HT$_{1A}$ receptor KO mice display increased anxiety-related and antidepressant-like behaviour would at first sight seem at odds with persuasive clinical evidence that anxiety and depression are commonly associated in patients with affective spectrum disorders. Regional variation in the expression of 5-HT$_{1A}$ receptors and other components of serotonergic transmission as well as the complex autoregulatory processes of 5-HT function which are operational in different brain areas may lead to a plausible hypothesis for this apparent contradiction. While few neuroadaptational changes were detected in 5-HT$_{1A}$ receptor KO mice, it is likely that more than a single component of serotonergic transmission is compromised in anxiety disorders or affective illness. For example, genetically driven impaired ability of the 5-HT transporter for rapid clearance of 5-HT following release into the synaptic cleft may elicit acute increases of 5-HT in the vicinity of serotonergic cell bodies and dendrites in the raphe complex and may exert a somatodendritic 5-HT$_{1A}$ receptor-mediated negative feedback that leads to lifetime abnormalities of serotonergic functioning (Lesch and Mössner, 1998). In combination with an impaired 5-HT$_{1A}$ receptor function, it is anticipated that compensatory changes would involve additional regulatory pathways, such as decreasing synthesis of increasing metabolism of 5-HT. This hypothesis could readily be tested in 5-HT$_{1A}$ receptor KO mice cross-bred with mice lacking the 5-HT transporter resulting in partial or complete 5-HT$_{1A}$/5-HT transporter double knockout (DKO) mice.

In view of these cross-species analogies and phenotypic similarities, these speculations are congruent with the demonstration that combined administration of SSRIs with 5-HT$_{1A}$ antagonists have led to earlier and enhanced therapeutic efficacy in depression (Artigas et al., 1996; Blier et al., 1997; Romero et al., 1996) compared to SSRI treatment alone. Thus, concurrent antagonism of 5-HT$_{1A}$ and/or 5-HT$_{1B}$ autoreceptors during 5-HT uptake blockade may have the potential to accelerate the antidepressant effect of 5-HT transporter inhibition. While this could be advantageous in antidepressant drug non-responders and is currently being explored as a concept for developing rapidly acting antidepressants, understanding the interaction between 5-HT$_{1A}$ receptors and other components of the 5-HT system may eventually help in the elaboration of optimized therapeutic approaches.

References


