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5-HT_{1A} receptor knockout mouse as a genetic model of anxiety

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Abstract

Low levels of the serotonin$_{1A}$ (5-HT_{1A}) receptor have been repeatedly found in mood and anxiety disorders. Stress often exacerbates psychiatric disease and can also reduce 5-HT_{1A} receptor levels. When receptor deficiency was produced in mice by genetic knockout, an anxiety-like phenotype was observed. Anxiety in mice is defined as a high level of avoidance of novel and unfamiliar environment and increased fear reaction. Other aspects of anxiety such as autonomic activation, increased stress responsiveness, and neuroendocrine abnormalities have also been described in receptor knockout mice. These data indicate that 5-HT_{1A} receptor knockout mice represent a genetic animal model of anxiety with both construct and face validities. Although the core phenotype of anxiety can be reproduced in knockout mice in various inbred and outbred backgrounds, abnormalities in 5-HT dynamics and resistance to the anxiolitic drug diazepam have been seen in one but not on other genetic backgrounds. This indicates that while the development of anxiety is an invariable consequence of receptor deficit, other features induced by receptor loss are strongly modulated by other gene(s). Strain-dependent variability within the core phenotype does not diminish the value of 5-HT_{1A} receptor knockout mice as a model of anxiety. Indeed, it is consistent with the manifestation of anxiety in genetically heterogeneous human population.

Keywords: Anxiety; Benzodiazepine; 5-HT (5-hydroxytryptamine, serotin); 5-HT receptor

1. The serotonin$_{1A}$ (5-HT_{1A}) receptor: biology and pharmacology

The 5-HT_{1A} receptor belongs to the superfamily of G-protein coupled receptors and is negatively coupled to adenylyl cyclase (Hoyer and Schoeffter, 1991; Julius, 1991). Among the 14 known 5-HT receptor subtypes, 5-HT_{1A} receptor received the most attention mainly because it is implicated in anxiety and partial 5-HT_{1A} receptor agonists are anxiolytics (Murphy, 1990; Barnes and Sharp, 1999; Olivier et al., 1999).

5-HT_{1A} receptors show an abundant and comparable expression in the brain of mammals, including humans. Brain 5-HT_{1A} receptors are located both pre- and postsynaptically (Hamon, 1997). Presynaptic 5-HT_{1A} receptors (5-HT_{1A} autoreceptors) are present on serotonergic neurons in the dorsal and medial raphe nuclei and provide a mechanism for the feedback inhibition of the 5-HT system. Activation of these receptors by 5-HT results in a reduction of the firing rate of the serotonergic neurons and suppression of 5-HT synthesis, 5-HT turnover, and 5-HT release in projection areas (Blier et al., 1987; Kennett et al., 1987; Sprouse and Aghajanian, 1988; Bohmaker et al., 1993; Jolas et al., 1993). Postsynaptic 5-HT_{1A} receptors are found at high density in limbic regions (such as hippocampus and septum) and in the frontal and enthorhinal cortices (Pazos and Palacios, 1985). Lower 5-HT_{1A} receptor levels are observed in amygdala. As in the case of presynaptic receptors, activation of postsynaptic 5-HT_{1A} receptors is generally believed to decrease the firing rate of postsynaptic cells (Sprouse and Aghajanian, 1988).

Effects of 5-HT_{1A} receptor selective ligands on animal behavior have been extensively studied. Partial agonists (buspirone, ipsapirone, gepirone) and at a certain degree full 5-HT_{1A} receptor agonists (8-hydroxy-2-(di-*n*-propylamino)tetralin, flesinoxan) result in an anxiolytic-like effect (Lucki et al., 1994; De Vry, 1995). There is a good correlation between the time and dose dependency of the anxiolytic effect, the inhibition of serotonergic firing in the dorsal raphe nuclei, and the inhibition of 5-HT release after systemic administration of 5-HT_{1A} receptor agonists (Jolas et al., 1995; Sommermeyer et al., 1993). It has been proposed that 5-HT_{1A} receptor agonists stimulate presynap-

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tic receptors, which inhibit 5-HT release and consequently reduce 5-HT signaling at target receptors such as postsynaptic 5-HT_{2A}, 5-HT_{2C}, and 5-HT_3 receptors.

The selective 5-HT_{1A} receptor antagonist WAY-100,635, by inhibiting the negative feedback, increases firing of the serotonergic neurons. However, this action has apparently no behavioral consequences (Olivier and Miczek, 1999). Consistent with these findings, microdialysis studies showed no detectable increase of 5-HT neurotransmission by 5-HT_{1A} receptor antagonists under normal conditions (Kreiss and Lucki, 1994). This suggests compensation in the 5-HT system, presumably mediated by 5-HT_{1B} receptors (see below). When given with selective 5-HT reuptake inhibitors, however, WAY-100,635 augments increases in 5-HT levels in terminal regions (Hjorth, 1993).

2. Functional deficit in 5-HT_{1A} receptor: risk factor in psychiatric disorders?

The availability of receptor specific ligands allowed monitoring the 5-HT_{1A} receptor in various disorders. A deficiency in the 5-HT_{1A} receptor has been reproducibly found in the limbic system in mood disorders (Lesch et al., 1992a; Meltzer and Maes, 1995). In a further study, decreased 5-HT_{1A} receptor binding was found in the brain of depressed suicide victims (Cheetham et al., 1990). Also, recent brain imaging studies using positron emission tomography (PET) have revealed decreased 5-HT_{1A} receptor densities in the medial temporal lobe and other limbic brain regions of patients with major depression (Drevets et al., 1999; Sargent et al., 2000). In addition, depressed patients showed blunted hypothalamic–pituitary–adrenal system responses following 5-HT_{1A} receptor agonist challenge that can also be interpreted as downregulation or hyporesponsiveness of postsynaptic 5-HT_{1A} receptors (Lesch et al., 1990). Downregulation of 5-HT_{1A} receptors in patients with major depression could not be reversed by the antidepressant fluoxetine (Sargent et al., 2000), raising the possibility that low receptor level is a trait characteristic of the disease. Reports also found 5-HT_{1A} receptor deficit in post-traumatic stress disorder and panic disorder (Mann, 1999; Lopez et al., 1998; Lesch et al., 1992a,b). Chronic stress, which is well known to be a major factor in the development of mood disorders, has also been shown to result in the down-regulation of 5-HT_{1A} receptors in the hippocampus of experimental animals (Watanabe et al., 1993; McKittrick et al., 1995; Fernandes et al., 1997; Meijer et al., 1997; Flugge, 1995; Lopez et al., 1998; Wissink et al., 2000). A hippocampal deficit in 5-HT_{1A} receptors could contribute to the cognitive abnormalities often seen in mood disorders (Rush et al., 1983; Bornstein et al., 1991; Ilsley et al., 1995; Tarbuck and Paykel, 1995). Taken together, these data indicate that low 5-HT_{1A} receptor levels may represent a risk factor in psychiatric disorders.

3. Modeling psychiatric disorder in mice by the genetic manipulation of 5-HT_{1A} receptor?

The possibility that receptor deficiency plays a role in mood and anxiety disorders (Mann, 1999; Lopez et al., 1998; Lesch et al., 1992a,b) prompted studies to genetically manipulate the 5-HT_{1A} receptor in mice. Gene expression can be conveniently downregulated or completely eliminated by knockout of one or both alleles of a gene. A receptor knockout mouse model may be considered to have construct validity because 5-HT_{1A} deficiency has been implicated in the development of psychiatric disorders.

In 1998, three groups, including our own, published the generation of 5-HT_{1A} receptor knockout mice (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998). Behavior of the knockout animals was tested in a variety of conditions evaluating fear, avoidance, conflict, and stress responsiveness. Subsequent reports tested learning, autonomic responses, status of the neuroendocrine system, and effect of various pharmacological agents on the behavior of 5-HT_{1A} receptor knockout mice.

4. Genetic inactivation of 5-HT_{1A} receptor in mice results in anxiety

All initial studies on the behavior of 5-HT_{1A} receptor knockout mice (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998) concluded an anxiety-like phenotype in these mice. Such consistency in behavioral data is rather remarkable considering that the three knockouts were generated on different genetic backgrounds in three different laboratories and tested under similar but not identical conditions. Beyond the strong influence of the genetic background, it has been reported that behavior of the same knockout mice can vary from laboratory to laboratory (Crabbe et al., 1999). Importantly, anxiety was apparent not only in homozygote but also in heterozygote 5-HT_{1A} receptor knockout mice, indicating that a partial receptor deficit is sufficient to elicit the phenotype. This raises the possibility that receptor downregulation is a risk factor in psychiatric disorders.

The claim that 5-HT_{1A} receptor knockout mice have an anxiety phenotype was based on tests evaluating behavioral response in conflict (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998). Conflict-induced fear and avoidance are common characteristics across anxiety disorders. The elevated plus maze (Lister, 1987) consists of a cross with opposing pairs of arms which are either open or enclosed. The maze is raised off the ground. The normal behavior of the animal is to stay in the enclosed compartment of the maze, which is less fearful. However, during normal exploratory activity, the animal will enter the open arms. To assess the anxiety level, number of entries into and time spent in the open arm are counted (Rodgers and Johnson, 1995). 5-HT_{1A} receptor knockout mice on both the inbred 129 sv and

outbred Swiss Webster (SW) backgrounds showed a reduction in open arm entries and time spent in the open arms (Table 1) (Ramboz et al., 1998; Sibille et al., 2000). Heisler et al. (1998) used elevated zero maze, a test apparatus which, in contrast to plus maze, has an annular runway divided into two open and two closed quadrants. In this test, 5-HT_{1A} receptor knockout mice on the inbred C57Bl6 background also displayed an anxiety phenotype indicated by the reduction in entries into and time spent in the open quadrants of the maze. Anxiety can also be assessed in the center of a brightly-lit open field (Treit and Fundytus, 1988). Animals tend to stay and move around the periphery of the field since the open area and bright light are aversive. The anxiety measured in an open field is assessed by both the time spent in the center of the open field and the number of entries into or the path length in this area. All of these measures were reduced in the three lines of 5-HT_{1A} receptor knockout mice, again supporting an anxiety-like phenotype (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998). Novel object test is another anxiety assay measuring the time, activity, and entrances around the novel object introduced in a familiar environment. A reduction in these measures was also reported in 5-HT_{1A} receptor knockout mice (Heisler et al., 1998). In a more recent study, Gross et al. (2002) used novelty-suppressed feeding as a measure of anxiety and found that food deprived 129 sv 5-HT_{1A} receptor knockout mice had a longer latency than controls to initiate feeding. Finally, 129 sv 5-HT_{1A} receptor knockout mice were found to spend more time "burying" and in stretch approach posture in a novel environment than wild-type controls, indicating a more 'anxious' behavioral structure (Olivier et al., 2001).

As the 5-HT_{1A} receptor is expressed at different neuronal compartments, it is of a considerable interest to know whether the pre- or post-synaptic receptor pool is required to maintain a normal level of fear and anxiety in mice. By using a conditional rescue approach, Gross et al. (2002) showed that expression of the 5-HT_{1A} receptor primarily in the hippocampus and cortex but not in the raphe nuclei is sufficient to rescue the behavioural phenotype of knockout mice. In summary, data indicate that deletion of the 5-HT_{1A} receptor in mice, specifically in forebrain structures, results in a robust anxiety phenotype. The phenomenological similarity between human anxiety and the phenotype of 5-HT_{1A} receptor knockout mice indicates a face validity of this animal model.

Table 1
Behavior of 5-HT_{1A} knockout mice on various genetic backgrounds in the elevated plus maze and open field tests

Behavioural test (place)	Swiss Webster (Cornell)	129 sv (Columbia/Utrecht)	C57Bl6 (UCSF/Cornell)
Elevated plus maze	↑	↑	↑ ↑
Open field	↑	↑	↑ ↑

5. Phenotype of 5-HT_{1A} receptor knockout mice: beyond the simple measures of anxiety

Most tests initially used for the behavioral characterization of 5-HT_{1A} receptor knockout mice were based on avoidance induced by conflict and fear, a common characteristic of anxiety disorders. Although this provides face validity for the 5-HT_{1A} receptor knockout mice as a model, avoidance is only one dimension of human anxiety. Other aspects include the activity of the autonomic systems, responsiveness to stress, the regulation of 5-HT dynamics, function of the neuroendocrine system, and neuronal excitability in circuitries involved in fear and anxiety.

5.1. Autonomic activation in 5-HT_{1A} receptor knockout mice

Anxiety patients, particularly those having panic attacks, experience increased heart rate and blood pressure. These are autonomic manifestations of anxiety and could be used in animal models to characterize and measure anxiety. Olivier et al. (2001) observed that in a novel environment (new cage), heart rate of 129 sv 5-HT_{1A} receptor knockout mice increased more than those of wild-type controls (200 vs. 100 bpm). Also, body temperature increased more significantly in knockout than control animals (2.5 vs. 1.5 °C). Baseline values of heart rate and body temperature were not different between the two groups of animals. Following an i.p. injection of saline, another stress situation, a similar difference was seen between knockout and wild-type mice (Olivier et al., 2001). Experiments were carried out with footshock that also demonstrated a greater heart rate increase in mutant than wild-type mice (Gross et al., 2000). All of these studies were carried out with receptor knockout mice on the 129 sv background. Similar studies with the other two lines of knockout mice will be necessary to assess the effect of the genetic background on these behaviors.

5.2. Increased stress responsiveness of 5-HT_{1A} receptor knockout mice

Hyperreactivity to various stressors is well known in anxiety disorders. When tested in a variety of behavioral paradigms, 5-HT_{1A} receptor knockout mice displayed increased stress responsiveness. Although the forced swim test was developed to screen for compounds with antidepressant activity (Porsolt et al., 1997), it is basically a stress situation in which escape-directed behavior (time in mobility) is measured in comparison to time spent in immobility. 5-HT_{1A} receptor knockout mice on both the SW and 129 sv backgrounds displayed more mobility (Parks et al., 1998; Ramboz et al., 1998). Tail suspension is a similar test and knockout mice on both the C57Bl6 and 129 sv backgrounds spent more time in mobility than wild-type animals (Heisler et al., 1998; Mayorga et al., 2001). This

behavior was reversed by pretreatment with alpha-methyl-*para*-tyrosine, but not by *para*-chlorophenylalanine, suggesting a role for enhanced catecholamine function (Mayorga et al., 2001).

There is however another interpretation for the behavior of 5-HT_{1A} receptor knockout mice in the forced swim and tail suspension tests. Since antidepressants induce a similar increase in behavioral response (characterized originally as decreased immobility), receptor knockout mice essentially behave as antidepressant-treated animals. The notion of antidepressant-like effect in knockout mice, however, could be misleading because neither the forced swim nor the tail suspension tests detect genuine antidepressant effect. A real antidepressant state can only be achieved with chronic drug treatment while a single dose of antidepressants elicits increased mobility in mice. Also, some drugs, effective in the behavioral tests, have no antidepressant profile. In summary, behavior of 5-HT_{1A} receptor knockout mice in the forced swim and tail suspension tests is more consistent with emotional reactivity and increased anxiety.

Finally, freezing elicited by aversive stimuli such as electric footshock is another measure of fear and anxiety (Fanselow, 1980). 5-HT_{1A} receptor knockout mice on the 129 sv background showed enhanced freezing as compared to controls (Gross et al., 2000), again indicating that mutant mice are more anxious. In summary, behaviors in various stress-related paradigms indicate increased stress responsiveness in all three 5-HT_{1A} receptor knockout lines.

5.3. Learning deficit in 5-HT_{1A} knockout mice

There is a high density of 5-HT_{1A} receptors in the hippocampus, suggesting that receptor knockout mice may have abnormalities in hippocampal functions such as learning and memory. Indeed, 5-HT_{1A} knockout animals on the SW genetic background showed a deficit in the hidden platform (spatial) version of the Morris water maze and the delayed version of the Y-maze (Sarnyai et al., 2000). Performance of knockout mice was not impaired in non-hippocampal memory tasks such as the visible platform (non-spatial) version of the Morris water maze, the immediate version of the Y-maze, and the spontaneous alternation test of working memory. Importantly, the selective 5-HT_{1A} receptor antagonist WAY-100,635 is not known to effect learning tasks (Meneses and Hong, 1999), suggesting that the knockout phenotype is not directly related to an acute receptor-loss but is rather due to long-term adaptive changes induced by the receptor-deficiency. These results suggest that reduced levels of hippocampal 5-HT_{1A} receptors could contribute to the development of some of the behavioral symptoms of mood disorders, which include deficits in declarative and episodic memory (Eichenbaum, 1999; Eichenbaum et al., 1999). However, these experiments were carried out with mutants on the SW background and it is currently not known whether cognitive defects are invariably associated with 5-HT_{1A} receptor deficiency in mice.

5.4. Regulation of 5-HT level in 5-HT_{1A} receptor knockout mice

The level of 5-HT in the synaptic and extrasynaptic space is controlled by the coordinated action of at least three factors. Firing of raphe neurons is controlled by 5-HT_{1A} autoreceptors located in the somatodendritic compartment of serotonergic neurons. Release of 5-HT at the terminal fields is regulated by the 5-HT_{1B} receptor. Once released, 5-HT is taken up by the 5-HT transporter located at the terminals of 5-HT neurons. It was predicted that removal of 5-HT_{1A} autoreceptors would affect extracellular levels of 5-HT. Extracellular levels of 5-HT can be estimated from brain dialysate levels. Interestingly, extracellular levels were not changed in SW and 129 sv 5-HT_{1A} receptor knockout mice as compared to matched wild-type mice in striatum and hippocampus (He et al., 2001; Knobelman et al., 2000, 2001a). Adaptive changes may explain that 5-HT_{1A} receptor deletion is without effect. For example, a compensatory increase in terminal 5-HT_{1B} autoreceptor activity could oppose increases in the firing of raphe neurons resulting in no net change in release. Indeed, Ramboz et al. (1998) have reported that suppression of [^{3}H]5-HT release by a 5-HT_{1B} receptor agonist is more pronounced in mesencephalic slices of 129 sv 5-HT_{1A} receptor knockout mice than in slices of wild-type animals. Similarly, there was a significantly greater reduction in extracellular 5-HT level in the striatum of 129 sv 5-HT_{1A} receptor knockout than control mice when challenged with a 5-HT_{1B} receptor agonist suggesting the development of enhanced sensitivity of striatal 5-HT_{1B} receptors (Knobelman et al., 2001a).

In contrast to 5-HT_{1A} receptor knockout mice on the SW and 129 sv backgrounds, dialysate 5-HT levels were significantly elevated in mutant animals on the C57Bl6 background both in the frontal cortex and hippocampus (Parsons et al., 2001). It is possible that there are no compensatory changes in 5-HT_{1B} receptor in mutants on the C57Bl6 background. It is also possible that C57Bl6 mice, especially the mutants, are sensitive to the microdialysis procedure and respond with an increase in 5-HT levels. Under stressful conditions during behavioral testing, levels of 5-HT are known to change (Vahabzadeh and Fillenz, 1994; Kirby et al., 1997; Hashimoto et al., 1999). We have recently noted that C57Bl6 mice, particularly of the 5-HT_{1A} receptor knockouts, are more anxious in the elevated maze and open field tests than matched SW mice indicating an increased emotionality of the C57Bl6 strain (Toth et al., unpublished). This is consistent with findings that C57Bl6 mice are more aggressive and susceptible to drugs of abuse than many other strains including 129 sv and SW.

5.5. Electrophysiological alterations in 5-HT$_{1A}$ knockout mice

Since 5-HT$_{1A}$ receptors are located both pre- and postsynaptically, their absence in receptor knockout mice could cause alterations in the neuronal firing and other electrophysiological properties of both serotonergic neurons and postsynaptic (for example hippocampal) neurons. Richer et al. (2002) investigated the activity of dorsal raphe neurons in 5-HT$_{1A}$ receptor knockout mice on the 129 sv background in brain slices. They found a nearly twofold increase in their mean firing rate, although 65% of the neurons was firing in their normal range. This however did not alter 5-HT (and norepinephrine) release, consistent with the microdialysis studies that demonstrated no change in the extracellular level of 5-HT in this line of mutant mice (Knobelman et al., 2001a,b). Sibille et al. (2000) and Sarnyai et al. (2000) studied the electrophysiological properties of the hippocampal network in 5-HT$_{1A}$ receptor knockout mice on the SW background. These studies revealed the absence of paired-pulse inhibition in the CA1 region and lack of paired-pulse facilitation in the dentate gyrus of mutant mice. These data indicate an abnormality in short-lasting neuroplasticity in 5-HT$_{1A}$ receptor knockout mice. Such abnormalities can have an impact on hippocampal excitability and learning and memory. Indeed, these mice displayed an increased limbic seizure susceptibility to kainic acid and cognitive defects (Sarnyai et al., 2000).

In summary, both the pre- and postsynaptic neurons in the 5-HT system show abnormalities in their electrophysiological properties consistent with the dual localization of the receptor. As these studies were conducted with single lines of receptor-deficient mice, the generalization of these finding awaits similar experiments in other lines of mutant mice.

5.6. The hypothalamic–pituitary–adrenal system in 5-HT$_{1A}$ knockout mice

Stress is accompanied with the activation of the hypothalamic–pituitary–adrenal system. Release of adrenocorticotropic hormone into the bloodstream by the pituitary triggers the secretion of corticosterone by the adrenal gland. Release of corticosterone induced by restrain and behavioral (novel environment) stress was partially blunted in 5-HT$_{1A}$ receptor knockout mice on the SW and 129 sv backgrounds (Sibille et al., 1998; Gross et al., 2000). Baseline levels were not different between mutant and control mice. These data indicate a dysregulation of the hypothalamic–pituitary–adrenal system following stress. Although different, dysregulation of the hypothalamic–pituitary–adrenal system is often seen in mood disorders and in some forms of anxiety. In depressed patients, an increased baseline cortisol and a decreased suppression of cortisol by dexamethason have been observed while decreased baseline cortisol and enhanced suppression have been documented in posttraumatic stress disorder (Plotsky et al., 1998; Yehuda et al., 1996).

6. 5-HT$_{1A}$ receptor knockout mice as a genetic model of anxiety

5-HT$_{1A}$ receptor-deficient mice are probably one of the most extensively studied receptor knockout animals. Beyond the behavioral characterizations, these mice were also tested for electrophysiological, neurochemical, and hormonal abnormalities. The majority of studies support the notion of an anxiety phenotype in 5-HT$_{1A}$ receptor-deficient mice.

Although some reports found 5-HT$_{1A}$ receptor-deficit in posttraumatic stress disorder and panic disorder, a reduced receptor level in human studies was mainly associated with depression (Mann, 1999; Lopez et al., 1998; Lesch et al., 1992a,b). Depression is often comorbid with anxiety and a deficit in 5-HT$_{1A}$ receptor may increase susceptibility to both depression and anxiety. Lack of signs of depression in receptor knockout mice may be due to the fact that depression, as we recognize it in humans, does not exist in mice. Nevertheless, certain aspects of a depressive state, such as cognitive and neuroendocrine abnormalities, can be recognized in mice. Indeed, defects in hippocampal memory and learning tasks were seen in SW 5-HT$_{1A}$ receptor knockout mice. Furthermore, regulation of the hypothalamic–pituitary–adrenal system was abnormal in 5-HT$_{1A}$ receptor knockout mice. Although the nature of the abnormality is different, depression is often associated with the dysregulation of the hypothalamic–pituitary–adrenal system.

Based on these data, we suggest that downregulation of the 5-HT$_{1A}$ receptor in both human and mouse increases susceptibility to a pathological condition that includes the symptoms of both anxiety and depression. In humans, depression is the principal finding mainly because of the severity of the associated clinical symptoms. In mice, anxiety is the predominant phenotype as anxiety-like behavior is relatively easy to recognize and measure and because mice lack core features of depression.

7. Effect of pharmacological agents in 5-HT$_{1A}$ knockout mice

Pharmacological challenges in knockout mice represent a powerful tool to better understand both the consequences of the knockout and the mechanism of drug action.

7.1. Altered responsiveness of 5-HT$_{1A}$ knockout mice to fluoxetine and other selective serotonin reuptake inhibitors

As described earlier, antidepressants elicit a reduction in immobility in tail suspension test that is predictive for the clinical effect of the drug. While in wild-type mice fluoxetine significantly decreased immobility, the drug had no effect in the 5-HT$_{1A}$ receptor knockout animals. Administration of selective 5-HT receptor antagonists in wild-type mice partially reproduced the phenotypes of the mutant

mice. Desipramine, a noradrenergic uptake inhibitor, decreased immobility in both wild-type and mutant mice. This suggests that 5-HT_{1A} receptors are required for the antidepressant-like activity of selective serotonin reuptake inhibitors as in the tail suspension test.

As selective serotonin reuptake inhibitors block 5-HT uptake, it was of interest to learn how such a pharmacological blockade, in combination with the genetic inactivation of the 5-HT_{1A} receptor, impacts the regulation of synaptic 5-HT levels. Systemic administration of fluoxetine evoked a more significant increase in extracellular 5-HT in all three 5-HT_{1A} receptor knockout lines as compared with matching controls in various brain regions (He et al., 2001; Knobelman et al., 2001b; Parsons et al., 2001). As pointed out earlier, deletion of the 5-HT_{1A} receptor alone does not alter 5-HT dynamics. Brain regions were differently affected by the loss of the 5-HT_{1A} receptor following treatment with a selective serotonin reuptake inhibitor and Knobelman et al. (2001b) suggested that the receptor plays a larger role in regulating 5-HT release in the striatum and possibly other brain regions innervated by the dorsal raphe nucleus (e.g. cortex) than in hippocampus innervated by the medial raphe nucleus. Similarly, Parsons et al. (2001) described a more significant dysinhibition of 5-HT release in the frontal cortex than ventral hippocampus in receptor knockout mice following treatment with selective serotonin reuptake inhibitors. The data demonstrating the significantly higher 5-HT level in fluoxetine-treated 5-HT_{1A} knockout mice indicate that the adaptive mechanisms that compensate for the lack of 5-HT_{1A} autoreceptors under normal physiological conditions are insufficient to oppose the increase in 5-HT levels caused by uptake inhibition. This is consistent with the notion that development of the antidepressant effect of selective serotonin reuptake inhibitors can be facilitated by the simultaneous blockade of presynaptic 5-HT_{1A} receptors (Artigas et al., 1996). It is believed that desensitization of the presynaptic 5-HT_{1A} receptors by long-term treatment with selective serotonin reuptake inhibitors and the concomitant increase in 5-HT levels are required for the development of the antidepressant action of these drugs (Hjorth, 1993; Artigas et al., 1996).

7.2. The anxiolytic activity of diazepam is dependent on the genetic background of 5-HT_{1A} knockout mice

Surprisingly, SW 5-HT_{1A} receptor knockout mice did not respond when injected with diazepam, a classical benzodiazepine with potent anxiolytic activity (Sibille et al., 2000). Recent studies demonstrated that these mutant mice are also resistant to the sedative effect of diazepam (Toth et al., unpublished data). The pharmacological effects of benzodiazepines are mediated by $GABA_A$ receptors, and further studies showed that binding of the benzodiazepine specific ligand methyl-[^{3}H]flunitrazepam was reduced both in the central nucleus and basolateral nucleus of amygdala (Sibille et al., 2000). The reduced flunitrazepam binding in amygdala is interesting because this region has been shown to be the main site of action for the anxiolytic effect of benzodiazepines in conflict-based behavioral assays such as the elevated plus maze (Graeff et al., 1993). $GABA_A$ receptors are pentameric channels and benzodiazepine receptors consist of mostly α, β and γ subunits (MacDonald and Olsen, 1994). The α subunit contains both the GABA and benzodiazepine recognition sites and the six different α subunits confer major pharmacological differences with respect of benzodiazepine, particularly in relation to anxiolytic effect (Pritchett et al., 1989). Molecular studies showed that both the α1 and α2 subunits were downregulated in the amygdala and cortex but not in the hippocampus or raphe of mutant mice (Sibille et al., 2000). These molecular changes can readily explain the reduced benzodiazepine binding and resistance of knockout mice to the anxiolytic and sedative effect of diazepam.

Table 2
Behavior of 5-HT_{1A} knockout mice on various genetic backgrounds following diazepam injection in the elevated plus maze and open field tests

Behavioural test following diazepam	Swiss Webster (Cornell)	129 sv (Utrecht)	C57Bl6 (Cornell)
Elevated plus maze	No effect	Anxiolytic	Anxiolytic
Locomotor activity in open field	No effect	Sedative	Sedative

In contrast to knockout line on the SW background, receptor-deficient mice on the 129 sv and C57Bl6 genetic backgrounds responded to diazepam (Table 2) (Olivier et al., 2001; Toth et al., unpublished). Also, no molecular change was detected in the $GABA_A$ complex in these two lines of mutant mice. These data clearly indicate that benzodiazepine resistance is mediated by an interaction between the 5-HT_{1A} receptor and one or more unknown genes, and that changes in the $GABA_A$ complex are independent of the anxiety phenotype. Knockout mice that showed benzodiazepine sensitivity were on inbred background while the benzodiazepine resistant knockouts were outbred. Inbreeding reduces the genome to single alleles and diminishes many gene interactions normally present in outbred populations. In terms of how a genetic alteration is manifested in the human population, experiments with outbred strains are more relevant. It will be interesting to learn whether 5-HT_{1A} receptor deficiency can be associated with benzodiazepine resistance seen in a group of patient with generalized anxiety.

8. Conclusion

5-HT_{1A} receptor-deficient mice represent a genetic animal model of anxiety with face and construct validities. Many of the core symptoms of anxiety can be recognized in 5-HT_{1A} receptor-deficient mice indicating that they will be extremely useful to decipher a pathogenic pathway leading to anxiety. Besides the robust anxiety phenotype which is

not affected by genomic variability, sensitivity/resistance to benzodiazepine in knockout mice is under the influence of a gene or a group of genes. This feature of the knockout may help to elucidate how genetic variability influences the appearance and severity of symptoms and the development of treatment-resistant anxiety.

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