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Research report

Differential effects of 5-HT_{1A} receptor deletion upon basal and fluoxetine-evoked 5-HT concentrations as revealed by in vivo microdialysis

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Abstract

An involvement of serotonin (5-HT) 1A receptors in the etiology of psychiatric disorders has been suggested. Hypo-responsiveness of the 5-HT_{1A} receptor is linked to anxiety and constitutive deletion of the 5-HT_{1A} receptor produces anxiety-like behaviors in the mouse. Evidence that 5-HT_{1A} receptor inactivation increases the therapeutic effects of antidepressants has also been presented. The present studies used in vivo microdialysis and homologous recombination techniques to examine the contribution of 5-HT_{1A} autoreceptors to these effects. Basal and fluoxetine-evoked extracellular concentrations of 5-HT were quantified in the striatum, a projection area of dorsal raphe neurons (DRN), of wild-type (WT) and 5-HT_{1A} receptor knock out (KO) mice. The density of 5-HT transporters was also determined. Basal 5-HT concentrations did not differ in WT and KO mice. Fluoxetine (10 mg/kg) increased 5-HT concentrations in both genotypes. This increase was, however, 2-fold greater in KO mice. In contrast, no differences in K^+-evoked 5-HT concentrations were seen. Similarly, neither basal nor stimulation-evoked DA differed across genotype. Autoradiography revealed no differences between genotype in the density of 5-HT transporters or post-synaptic 5-HT_{2A} receptors, an index of 5-HT neuronal activity. These experiments demonstrate that, under basal and KCl stimulated conditions, adaptive mechanisms in the 5-HT system compensate for the lack of 5-HT_{1A} autoreceptor regulation of DRN. Furthermore, they suggest that the absence of release-regulating 5-HT_{1A} autoreceptors in the DRN can not account for the anxiety phenotype of KO mice. The enhanced response to fluoxetine in KO mice is consistent with pharmacological studies and suggests that adaptive mechanisms that occur in response to 5-HT_{1A} receptor deletion are insufficient to oppose increases in 5-HT concentrations produced by acute inhibition of the 5-HT transporter. Published by Elsevier Science B.V.

Theme: Neurotransmitter, modulators, transporters and receptors

Topic: Serotonin

Keywords: 5-Hydroxytryptamine; 5-Hydroxytryptamine$_{1A}$ receptor; Dialysis; Fluoxetine; Knockout

1. Introduction

Several laboratories [19,28,34,38] have shown that mice lacking the 5-HT_{1A} receptor exhibit anxiety-like behavior in different experimental models indicating that hypofunction of the 5-HT_{1A} receptor leads to anxiety. Indeed, hyporesponsiveness of the 5-HT_{1A} receptor is associated with panic disorder, one form of anxiety [25]. The role, however, of pre- versus post-synaptic 5-HT_{1A} receptors in the etiology of anxiety is unclear.

An involvement of 5-HT_{1A} receptors in the delayed onset of action of selective serotonin uptake inhibitors

Abbreviations: DA, dopamine; DRN, dorsal raphe nuclei; 5-HT, 5-hydroxytryptamine; KO, knockout; LSD, lysergic acid diethylamide; MRN, medial raphe nuclei; SSRI, selective serotonin reuptake inhibitor; WT, wild-type

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(SSRIs) and other antidepressants has been suggested. It has been hypothesized that desensitization of 5-HT_{1A} autoreceptors and the resulting enhancement of SSRI-evoked 5-HT concentrations are required for the antidepressant action of SSRIs [2,22,31]. Indeed, the partial 5-HT_{1A} agonist, (−)-pindolol, potentiates SSRI-induced increases in extracellular 5-HT concentrations and accelerates their antidepressant effects [2,31]. These actions have been attributed to the blockade of 5-HT_{1A} autoreceptors and enhanced 5-HT concentrations in projection areas of 5-HT neurons. Furthermore, the acute administration of pindolol, which functions at least in part as a 5-HT_{1A} receptor antagonist, also enhances SSRI-evoked increases in extracellular 5-HT concentrations and accelerates their antidepressant effects [2,31]. Since, however, pindolol also functions as a β-adrenergic receptor antagonist as well as an antagonist of 5-HT_{1A} post-synaptic receptors [2,3,21], fundamental questions exist as to whether the effects of this agent result solely from interactions with 5-HT_{1A}autoreceptors. It is also unclear as to whether compensatory mechanisms develop as a consequence of chronic 5-HT_{1A} receptor inactivation and whether their development prevents increases in 5-HT neurotransmission produced by 5-HT_{1A} receptor blockade.

5-HT_{1A} autoreceptors are located on 5-HT neurons in the dorsal and medial raphe nuclei (DRN and MRN) and provide feedback regulation of the 5-HT system [18]. Their activation reduces the firing of 5-HT neurons, suppresses 5-HT synthesis, and reduces 5-HT turnover and release in projection areas of the raphe that include the frontal cortex, hippocampus, and striatum [6,7,22–24]. Post-synaptic 5-HT_{1A} receptors are highly expressed in hippocampus, lateral septum, and the frontal and entorhinal cortex [30]. Lower concentrations are observed in amygdala, thalamic and hypothalamic nuclei, and the lateral striatum.

The goals of the present studies were twofold. Since presynaptic 5-HT_{1A} receptors regulate 5-HT neurotransmission [12,21], we sought to determine whether the lack of presynaptic receptors in 5-HT_{1A} receptor knock out (KO) mice is associated with increases in the activity of 5-HT neurons. Secondly, we sought to determine whether the absence of these receptors results in an enhanced response of 5-HT neurons to the SSRI, fluoxetine. In vivo microdialysis was used to measure basal and stimulus-evoked 5-HT overflow in the dorsal striatum, a terminal projection area of DRN neurons, in KO and WT mice. DRN neurons were evaluated since a study in rats suggest that they may be more sensitive to 5-HT_{1A} receptor-mediated inhibition than neurons originating in the MRN [35]. Since, previous studies have indicated a role of 5-HT neurons in regulating mesostriatal dopamine (DA) neurotransmission [4], DA concentrations were also monitored. The density of 5-HT transporters and of 5-HT_{2A} receptors was also determined in order to further characterize changes in 5-HT neurotransmission that occur following 5-HT_{1A} receptor deletion.

2. Materials and methods

2.1. Animals

5-HT_{1A} KO mice were generated by homologous recombination as previously described [28]. The chimeras were backcrossed to Swiss-Webster mice to obtain heterozygotes (129^{sv}×Swiss-Webster). These F1 animals were crossbred to produce homozygous F2 mutants [28]. Control 129^{sv} and Swiss-Webster mice were similarly bred. To avoid a disequilibrium of genes that are linked to the mutation, WT F2 progeny with two WT 129^{sv} 5-HT_{1A} receptor alleles were selected by single strand length polymorphism [28,41]. By using this method, we were able to generate control mice that matched the homozygous mice in background except that their 5-HT_{1A} receptor gene was not inactivated. Experiments were conducted in accordance with the guidelines established by the Institutional Care and Use Committee of the Intramural Research Program of the National Institute on Drug Abuse (NIDA), National Institutes of Health, and the Cornell Institutional Animal Care and Use Committee. The facilities were fully accredited by the American Association for the Accreditation of Laboratory Animal Care. Mice were housed one per cage and maintained under a constant temperature (25°C) and a 12:12 h light/dark cycle (lights on 06:00 h). Food and water were available ad libitum.

2.2. In vivo microdialysis

Male WT and 5-HT_{1A} KO mice (6–8 weeks old) were anesthetized with ketamine (80 mg/kg) and xylazine (8.0 mg/kg) and placed in a stereotaxic apparatus for insertion of a microdialysis guide cannula (CMA 11: CMA Inc., Nagog, MA) into the dorsal striatum as described previously [41]. The coordinates (mm from bregma) were: AP: −1.8, ML: ±3.8, DV: 4.4 [40]. Four days after cannula implantation, the mice were gently restrained and a microdialysis probe (2 mm membrane, 6000 mol. wt. cutoff; CMA 11, CMA Inc.) was inserted into the guide cannula. The inflow line to the probe was connected to a microinfusion pump (Harvard 22, Harvard Apparatus, South Natick, MA) via a liquid swivel (quartz lined) and wire tether. The tether was attached at one end to the liquid swivel and, at the other end, to a pedestal embedded in the cranial cement stage on the head. The swivel tether assembly (Instech Laboratories Inc., Plymouth Meeting, PA) was hung from the top of the cage by a balance arm (Instech Laboratories) to allow free movement of the mouse in the cage. The outflow tubing followed the same path as the inflow tubing, ending at the swivel inside a microcentrifuge tube. Artificial cerebral spinal fluid (aCSF) containing 145 mM NaCl, 2.8 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 5.4 mM D-glucose, and 0.25 mM ascorbic acid (pH 7.2–7.4) was perfused through the microdialysis

probe at a rate of 0.3 μl/min for an equilibration period of 8 h. The flow rate was increased to 0.6 μl/min, 2 h prior to the first sample collection, and remained at this rate for the experiment. Four consecutive 30 min dialysate samples were collected for determination of basal concentrations of 5-HT and DA. The aCSF was then replaced with that containing 60 mM KCl for 30 min. Three consecutive 30 min samples were then collected. Additional animals received an i.p. injection of fluoxetine (10.0 mg/kg) or saline and four 30-min dialysate samples were collected. Following completion of experiments, animals were euthanized and their brains removed for histological verification of probe placements. Only animals with probes confined to the dorsal striatum were used for subsequent data analysis.

2.3. Chromatographic analysis

The 5-HT and DA content of dialysate samples were analyzed using HPLC coupled to electrochemical detection. Chromatographic separations were performed with a microbore HPLC column (C18: i.d. 100×1 mm; 3 μm; BAS, West Lafayette, IN) in conjunction with a dual piston pump (PM 80; BAS, West Lafayette, IN). A six-port rotary valve (Model 7125, Rehodyne, Berkeley, CA) was used for sample injection. The mobile phase consisted of 25 mM NaH_2PO_4, 100 μM ethylenediaminetetraacetate, 3.9 mM sodium octyl sulfate, 8% methanol (v/v), 0.6% tetrahydrofuran (v/v), and an apparent pH of 5.0. The flow rate of the mobile phase was 100 μl/min. Electrochemical detection was accomplished using a BAS LC-4C amperometric detector. The working electrode was set at +650 mV versus Ag/AgCl. Output from the detector was recorded on a dual pen chart recorder. Standard curves were constructed for each analyte and used to quantify concentrations in dialysate samples. The detection limits for 5-HT and DA (signal/noise ratio: 3:1) were 0.25 and 0.5 nM, respectively.

2.4. 5-HT transporter and 5-HT_{2A} receptor autoradiography

Brains were removed and kept at −80°C until assayed. Coronal sections were cut with a microtome cryostat. Consecutive superimposable sections were used to determine total and nonspecific binding. 5-HT transporter binding was measured by using 1.5 nM [^{3}H]cyano-imipramime (American Radiolabeled Chemical Inc., St. Louis, MO). [^{3}H]Cyano-imipramime rather than [^{3}H]paroxetine was used in view of the higher specific activity of the former ligand [16,38]. Sections were incubated overnight at +4°C in a buffer containing 50 mM Tris–HCl and 150 mM NaCl (pH 7.4). Nonspecific binding was determined in the presence of 2 μM fluoxetine. Sections were exposed to Hyperfilm (Amersham Pharmacia, Piscataway, NJ) for 4 weeks. 5-HT_{2A} receptor binding was carried out according to a published procedure [16,38]. ^{125}I-Labeled lysergic acid diethylamide (LSD, 50 pM) (NEN, Boston, MA) was used in the presence of 0.2 nM haloperidol to reduce marginal LSD binding to dopamine D_2 receptors. Sections were incubated 1 h at room temperature in 50 mM Tris–HCl buffer (pH 7.4). Nonspecific binding was determined in the presence of 200 nM spiperone. Sections were exposed overnight. Computerized densitometry was performed with the NIH Image program. Quantification was based on a series of [^{3}H] and [^{125}I] autoradiographic internal standards (Amersham Pharmacia, Piscataway, NJ). Seven mice were examined in each group.

2.5. Data analysis

The Student's *t*-test was used to compare basal DA and 5-HT concentrations (nM) in WT and KO mice. A two-factor (genotype×K^+) analysis of variance (ANOVA) was used to analyze the effects of K^+. A two-factor (genotype×time) repeated-measures analysis of variance was used to analyze the effects of acute administration of fluoxetine and saline on dialysate concentrations. Simple effects tests or pairwise group comparisons were used to probe significant differences identified by ANOVA. The Student's *t*-test was used to analyze autoradiographic data. Statistical significance was assumed at $P<0.05$.

2.6. Drugs

Fluoxetine hydrochloride was a gift from Lilly Pharmaceuticals (Indianapolis, IN). Haloperidol and spiperone were obtained from RBI–Sigma (Natick, MA). Drugs were dissolved in water (fluoxetine) or ethanol (haloperidol and spiperone, 5 mg/ml stock solution) and injected i.p. in sterile saline (0.9% NaCl).

3. Results

Dialysate concentrations of a neurotransmitter provide an estimate of extracellular concentrations [29]. Therefore, in vivo microdialysis was used to determine if loss of somatodendritic 5-HT_{1A} receptors alters basal 5-HT concentrations in the dorsal striatum, a terminal projection area of DRN neurons. In view of the previously documented interactions of 5-HT and DA neurons [14], DA concentrations were also quantified. Despite the lack of presynaptic 5-HT_{1A} receptors in KO mice, no difference between genotype in basal dialysate 5-HT concentrations was observed ($t(1,27)=0.93$; $P=0.9$). Similarly, basal DA concentrations did not differ in KO as compared to WT mice ($t(1,28)=0.8$; $P=0.4$).

KCl was perfused through the dialysis probe in order to determine whether the depolarization-evoked release of 5-HT or DA differs between genotypes (Fig. 1). The

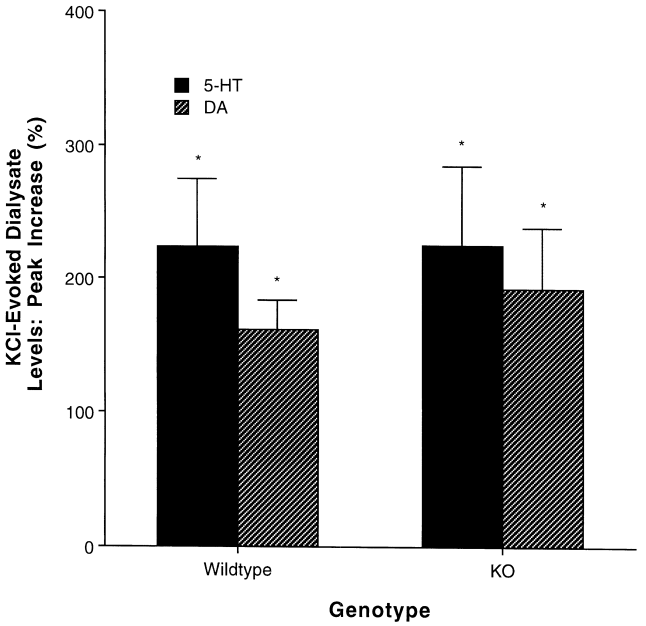

Fig. 1. Depolarization-evoked release of 5-HT in WT (n=15) and KO (n=14) animals. aCSF containing 60 mM KCl was perfused through the dialysis probe for 30 min. The data are expressed as the maximal increase (mean±S.E.M.) relative to basal concentrations. Asterisks indicate significant increase from basal concentrations.

addition of 60 mM KCl to the perfusate significantly increased 5-HT concentrations in both WT and KO mice (F(1,25)=31.4; P=0.001). There was no difference between genotype in the magnitude of these effects (F(1,25)=0.8; P=0.4). Similarly, K^+-evoked DA concentrations were similar in the two genotypes. ANOVA revealed a significant effect of K^+ (F(1,8)=7.6; P=0.03) but no significant effect of genotype (F(1,8)=2.9; P=0.1).

Pharmacological studies indicate an important role of presynaptic 5-HT_{1A} receptors in opposing SSRI-evoked increases in 5-HT concentrations [2]. Fig. 2 shows that acute administration of the prototypic SSRI, fluoxetine (10 mg/kg; i.p), increased 5-HT concentrations in both KO and WT mice. However, the magnitude of this effect was significantly greater in KO mice. ANOVA revealed significant effects of genotype (F(1,19)=4.4; P=0.05) and time (F(4,19)=16.8; P=0.001) and a significant genotype× time interaction (F(4,19)=3.9; P=0.05). In contrast to fluoxetine, the acute administration of saline did not modify 5-HT concentrations in either genotype (genotype: F(1,10)=0.7; P=0.8; time: F(4,40)=0.6; P=0.7) (data not shown).

Fig. 3 shows the influence of acute fluoxetine administration upon DA concentrations in the striatum. In contrast to 5-HT, fluoxetine failed to modify DA concentrations in KO or WT mice. ANOVA revealed no significant effect of genotype (F(1,21)=0.05; P=0.9) or time (F(4,84)=0.5; P=0.8) on DA concentrations.

To determine whether the loss of presynaptic 5-HT_{1A} receptors is associated with an alteration in the 5-HT

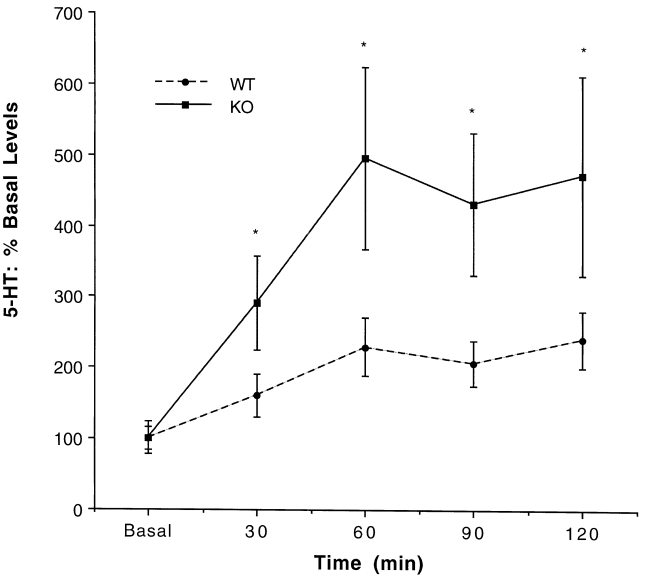

Fig. 2. Influence of acute fluoxetine administration (10 mg/kg) on 5-HT concentrations in WT (n=12) and KO (n=9) animals. Data are expressed as a percentage of basal concentrations. Ordinate indicates time post injection. Asterisks indicate significant difference between genotype.

transporter, [^{3}H]cyano-imipramine binding was quantified in the striatum. No difference between KO and WT mice in the binding of [^{3}H]cyano-imipramine to the 5-HT transporter was seen (P=0.10). Similarly, no difference in 5-HT transporter binding was observed in the DRN, MRN or terminal projection areas of these nuclei (Table 1). Table 2 shows that the binding of [^{125}I]LSD to 5-HT_{2A} receptors is unaltered in either the striatum or cortex of KO mice.

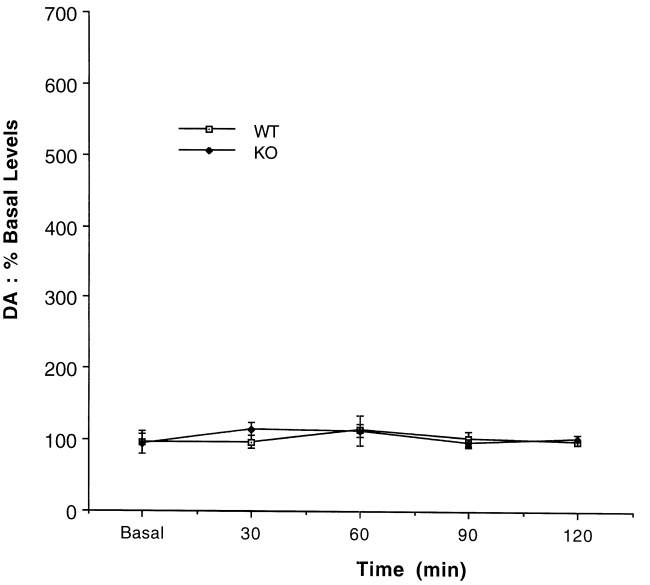

Fig. 3. Influence of acute fluoxetine administration (10 mg/kg) on DA concentrations in WT (n=12) and KO (n=11) animals. Ordinate: DA concentrations (nM). Abscissa: time post-injection (min).

Table 1
[^{3}H]Cyano-imipramine binding in WT (n=7) and KO (n=7) mice as an indicator of 5-HT transporter concentrations[a]

	WT	KO	
Prefrontal cortex	12.46±0.55	12.46±0.71	P=0.5
Frontal cortex	20.13±1.21	21.01±0.78	P=0.5
Hypothalamus	48.11±0.52	45.34±1.01	P=0.2
Amygdala (BLA)	33.05±1.21	33.95±0.85	P=0.5
Hippocampus (CA3)	19.92±0.91	18.40±1.02	P=0.3
Dorsal raphe	61.04±1.78	57.73±2.81	P=0.2
Median raphe	87.45±4.68	87.14±5.80	P=0.6
Central gray	32.34±0.82	30.37±0.63	P=0.1
Striatum	25.84±1.09	25.02±1.52	P=0.1

[a] Values are in fmol/mg wet tissue.

4. Discussion

These studies demonstrate that the ability of the SSRI, fluoxetine, to increase 5-HT concentrations in the dorsal striatum is enhanced following constitutive deletion of the 5-HT$_{1A}$ receptor. In contrast, the absence of 5-HT$_{1A}$ receptors does not modify basal or depolarization-evoked 5-HT concentrations, or the density of 5-HT transporters. Similarly, basal and stimulus-evoked DA concentrations are unaltered in the striatum following deletion of this receptor type.

Targeted inactivation of the 5-HT$_{1A}$ receptor in mice results in anxiety [19,28,34]. Since many pharmacological agents that increase 5-HT neurotransmission produce anxiety-like behavior in different experimental models [15,17], it has been hypothesized that the anxiety phenotype of KO mice is due to the absence of 5-HT$_{1A}$ autoreceptors and the resulting increase in 5-HT neurotransmission in terminal projection areas of raphe neurons. Indeed, evidence that 5 HT$_{1A}$ receptors inhibit the firing of DRN 5-HT neurons in the mouse has recently been presented [11]. If, in fact, this is the case, then extracellular concentrations of 5-HT as well as the depolarization-evoked release of 5-HT should be increased in KO as compared to WT mice.

Microdialysis studies revealed no alteration in basal 5-HT concentrations in the striatum of KO mice. Since dialysate concentrations of a neurotransmitter provide an estimate of extracellular concentrations [9,29], these findings indicate that extracellular concentrations of 5-HT are unaltered following constitutive deletion of the 5-HT$_{1A}$ receptor. The ability of K^+ to increase dialysate 5-HT concentrations also did not differ between genotype, indicating that the depolarization-evoked release of 5-HT did not differ between WT and KO mice. The failure of constitutive deletion of the 5-HT$_{1A}$ receptor to alter basal or depolarization-evoked dialysate 5-HT concentrations suggest that the absence of 5-HT$_{1A}$ autoreceptors and disinhibition of 5-HT neurons projecting to the striatum can not account for the anxiety phenotype of KO mice. In this regard, however, it is important to note that dialysis studies were conducted in the dorsal striatum, a region innervated by DRN neurons [30]. Therefore, increases in 5-HT neurotransmission may occur in projection regions (e.g. hippocampus, amygdala) of MRN and these may underlie the increased anxiety of KO mice. Alternatively, genotype-dependent difference in 5-HT neurotransmission may only become apparent following exposure of animals to stressful stimuli.

Table 2
[^{125}I]LSD binding in WT (n=7) and KO (n=7) mice as an indicator of 5-HT$_{2A}$ receptor concentrations[a]

	WT	KO	
Frontal cortex	49.4±5.7	47.8±1.6	P=0.8
Parietal cortex	38.9±2.7	36.5±3.7	P=0.6
Striatum	68.8±1.3	66.2±1.1	P=0.2

[a] Values are in fmol/mg of wet tissue.

Recent studies have shown that alterations in 5-HT uptake by the 5-HT transporter can modify the extraction fraction of 5-HT from the dialysis probe leading to over or under estimations of true extracellular concentrations [9,29,42]. Pharmacological manipulations that increase uptake increase the extraction fraction of the probe leading to an increase in dialysate concentrations. In contrast decreases in uptake decrease these parameters. Therefore, the possibility arises that alterations in 5-HT uptake may occur as a consequence of 5-HT$_{1A}$ receptor deletion and these changes may mask increases in extracellular concentrations that occur in response to 5-HT$_{1A}$ receptor deletion. Although this hypothesis was not evaluated in the present study, autoradiography revealed no alterations in the density of 5-HT transporters in the striatum or other projection areas of raphe nuclei. These findings indicate that 5-HT transport capacity does not differ in KO mice.

Previous studies have shown that 5-HT$_{2A}$ receptors are down-regulated following pharmacological treatments that increase 5-HT release [5,32]. Therefore, differences in the density of these receptors provide an assessment of post-synaptic receptor function as well as an indirect measure of changes in 5-HT neuronal activity. The finding that 5-HT$_{2A}$ receptor density was unchanged in projection regions of DRN neurons is consistent with a lack of change of presynaptic 5-HT neurotransmission in the dorsal striatum of KO mice. Furthermore, they indicate that the function of post-synaptic 5-HT$_{2A}$ receptors is also unaltered following 5-HT$_{1A}$ receptor deletion.

Both in vitro and in vivo studies have shown that 5-HT facilitates DA release in the striatum [4,10,43]. No significant differences in basal or depolarization-evoked DA concentrations were observed in KO and WT mice. The lack of change of DA concentrations in KO mice is important in that it suggest that mesostriatal DA neurotransmission in unaltered following 5-HT$_{1A}$ receptor deletion. Furthermore, it provides additional, albeit indirect, evidence that presynaptic 5-HT function is unaltered in the striatum of KO mice.

5-HT_{1A} receptor antagonists prevent decreases in extracellular 5-HT concentrations produced by acute agonist administration [24]. However, they do not, by themselves, modify 5-HT concentrations [1,13,37]. Therefore, alterations in 5-HT or DA neurotransmission that occur as a consequence of 5-HT_{1A} deletion may only be detectable under conditions of increased receptor activation. Studies, however, comparing depolarization-evoked 5-HT concentrations in WT and KO mice revealed no differences between genotype. Similarly, no difference in DA concentrations were seen. These data strongly suggest that the depolarization-induced release of 5-HT as well as DA is unaffected by 5-HT_{1A} receptor depletion. They are also in line with a recent study which showed no effect of 5-HT_{1A} receptor inactivation on the electrically-evoked release of [^{3}H]5-HT from mesencephalic slides [34]. Taken together, these finding strongly suggest that the lack of 5-HT_{1A} autoreceptors regulating the activity of DRN neurons is not the critical abnormality underlying the increased anxiety observed in KO mice. Rather they suggest that the loss of 5-HT_{1A} receptor-mediated feedback control of MRN neurons or the deletion of post-synaptic 5-HT_{1A} receptors in terminal regions of DRN or MRN mediate the anxiety phenotype of KO mice. Evidence in support of the latter hypothesis has recently been presented [38].

An explanation for the lack of change of presynaptic 5-HT function in KO mice is lacking. In contrast to receptor antagonists, gene knockout techniques inactivate receptors from embryonic life. Therefore, adaptive changes most likely underlie the apparently normal presynaptic 5-HT function of KO mice. In this regard, it is important to note that compensatory increases in terminal 5-HT_{1B} autoreceptor activity or sensitivity would oppose increases in the firing of raphe neurons resulting in no net change in release. Interestingly, Ramboz et al. [34] have reported that the inhibition of [^{3}H]5-HT release produced by a 5-HT_{1B} receptor agonist is more pronounced in mesencephalic slices of 5-HT_{1A} receptor KO mice than in slices of WT animals. Therefore, sensitization of 5-HT_{1B} receptors in KO mice may, at least partly, compensate for 5-HT_{1A} autoreceptor loss. Alternatively, presynaptic 5-HT_{1A} receptors may not be tonically active. If such was the case, their absence in KO mice would cause no change in 5-HT release. Although neurochemical data provide support for this hypothesis, several studies have shown that the firing rate of raphe neurons in increased in response to the selective 5-HT_{1A} receptor antagonist WAY 100,635 [12,13,27].

An involvement of 5-HT_{1A} autoreceptors in delaying the therapeutic effects of SSRIs and other antidepressants has previously been suggested [2]. Elimination of 5-HT_{1A} receptor-mediated feedback inhibition increases SSRI-induced increases in 5-HT concentrations [2]. It has been hypothesized that 5-HT_{1A} autoreceptor desensitization and the resulting enhancement of SSRI-evoked 5-HT concentrations are required for the antidepressant action of SSRIs [2,20]. The present microdialysis studies provide additional evidence that the ability of SSRIs to increase extracellular 5-HT concentrations is increased following 5-HT_{1A} receptor inactivation. In agreement with previous studies rat [23,36], the acute administration of fluoxetine to WT mice significantly increased 5-HT concentrations relative to basal values. An increase in 5-HT concentrations was also observed in KO mice. However, the magnitude of this effect was over 2-fold greater than that in WT mice. These data are noteworthy. They demonstrate that the adaptive mechanisms that maintain normal 5-HT neurotransmission under physiological conditions are insufficient to oppose the increase in 5-HT concentrations caused by transporter inhibition [2]. In addition, they indicate that compensatory mechanisms that occur following prolonged 5-HT_{1A} receptor inactivation (e.g. 5-HT_{1B} autoreceptor up-regulation) will be insufficient to oppose the increase in 5-HT concentrations produced by combined SSRI and 5-HT_{1A} receptor antagonist treatment. In contrast to 5-HT, the systemic administration of fluoxetine did not modify DA concentrations in the dorsal striatum of WT mice. This finding is in line with previous data obtained in the rat [8,26,33]. Fluoxetine was also ineffective in modifying DA concentrations in KO mice, suggesting that the enhanced response of DR neurons to 5-HT_{1A} receptor deletion is selective.

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