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Modification of serotonin neuron properties in mice lacking 5-HT_{1A} receptors

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Abstract

Using null mutant mice for the 5-HT_{1A} receptor (5-HT_{1A} −/−), extracellular electrophysiological recordings were first conducted to evaluate the impact of its genetic deletion on the firing rate of dorsal raphe 5-hydroxytryptamine (5-HT) neurons. Experiments were also done using brain slices to assess whether any compensation phenomenon had taken place in key receptors known to control 5-HT and norepinephrine release. The mean firing rate of 5-HT neurons was nearly doubled in 5-HT_{1A} −/− mice, although 65% of the neurons were firing in their normal range. In preloaded brain slices, the 5-$HT_{1D/B}$ receptor agonist sumatriptan equally inhibited the electrically evoked release of [^{3}H]5-HT in mesencephalic slices (containing the dorsal and median raphe) from wildtype and 5-HT_{1A} −/− mice. The 5-HT_{1B} receptor agonist CP 93129 (1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*-pyrrol (3, 2-*b*) pyridin-5-one) and the α_2-adrenoceptor agonist UK14,304 (5-bromo-*N*-(4, 5-dihydro-1*H*-imidazol-2-yl)-6-quinoxalinamine) produced the same inhibitory effect in both groups of mice in hippocampus and frontal cortex slices. No difference was observed on the UK14,304-mediated inhibition of [^{3}H]norepinephrine from preloaded slices of the two latter structures between the two groups of mice. In conclusion, the loss of control of the 5-HT_{1A} autoreceptor in 5-HT_{1A} −/− mice lead to a significant enhancement of 5-HT neuronal firing, but it did not alter 5-HT or norepinephrine release in any of the brain structures examined. In addition, it was not associated with changes in the function of 5-HT_{1D} and 5-HT_{1B} autoreceptors and of α_2-adrenergic heteroreceptors on 5-HT neurons, nor of that of α_2-adrenoceptors on norepinephrine terminals.

Keywords: 5-HT_{1A} receptor mutant mouse; Autoreceptor; Heteroreceptor; Cortex, frontal; Hippocampus; Raphe

1. Introduction

The 5-HT_{1A} receptors are $G_{i/o}$ protein-coupled receptors, located on the cell body and dendrites of 5-HT neurons and on postsynaptic neurons with a particularly high density in limbic structures. These receptors mediate an inhibitory effect on firing rate through K^+ channels. They can also be coupled to Ca^{2+} channels and second messenger systems (Jacobs and Azmitia, 1992).

The therapeutic response of several antidepressant drugs like selective serotonin reuptake inhibitors may rely in part on the desensitization of 5-HT_{1A} autoreceptors, via the partial loss of the negative feedback action normally exerted by this autoreceptor on 5-hydroxytryptamine (5-HT) neuronal firing (Blier and De Montigny, 1994). Other antidepressant treatments, like tricyclic antidepressants and electroconvulsive shock treatment, increase the sensitivity of postsynaptic 5-HT_{1A} receptors. Nevertheless, a net effect of all major classes of antidepressant treatments on 5-HT transmission is an increase in the degree of activation of postsynaptic 5-HT_{1A} receptors, at least in laboratory animals (Haddjeri et al., 1998). Consistent with the proposed mechanism of action of selective serotonin reuptake inhibitors in major depression, the 5-HT_{1A} receptor antagonist pindolol has been shown to shorten the onset of action of 5-HT reuptake inhibitors in 8 of 10 double-blind studies by decreasing the inhibitory feedback on 5-HT neuron firing rate (Berman et al., 1999; Isaac, 2001; Bordet et al., 1998; Maes et al.,1996, 1999; Perez et al., 1997; Tome et al., 1997; Zanardi et al., 1997, 1998, 2001). This leads rapidly to enhanced synaptic levels of 5-HT in the presence of 5-HT reuptake blockade (Artigas et al., 1996; Blier and Bergeron, 1998). Moreover, 5-HT_{1A} receptor agonists like buspirone

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and gepirone can be used, alone or in combination with antidepressant drugs, to treat major depression and generalized anxiety disorder (Bouwers and Stein, 1997; Robinson et al., 1990; Wilcox et al., 1996).

The neurotransmitter norepinephrine can also play an important role in the mechanism of action of antidepressants (Anand and Charney, 2000). Moreover, the 5-HT and norepinephrine neurotransmitter systems are endowed with reciprocal interactions throughout the brain (Mongeau et al., 1997). For example, the α_2-adrenoceptor agonist clonidine decreases 5-HT neuronal firing and release as a result of its inhibitory effect on noradrenergic neuronal function (Tao and Hjörth, 1992). In contrast, the α_2-adrenoceptor antagonist mirtazapine would exert its antidepressant action by enhancing norepinephrine release, and indirectly that of 5-HT as well, after long-term administration (De Boer, 1995; Haddjeri et al., 1997a). Conversely, a three-week but not a subacute treatment with serotonin selective reuptake inhibitors attenuates the firing rate of noradrenergic neurons (Szabo et al., 1999, 2000).

Null mutant animals for the 5-HT_{1A} receptor (5-$HT_{1A}-/-$) have been produced and shown to be more anxious in different behavioral tests (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998). Given the prior demonstration that 5-HT_{1A} autoreceptor function is absent in 5-$HT_{1A}-/-$ mice (Ramboz et al., 1998), this study was thus undertaken to evaluate the impact of this genetic deletion on 5-HT neuronal firing activity and to determine if any compensation phenomenon had taken place in presynaptic receptors controlling 5-HT and noradrenergic neuronal function in the mutants. To this end, extracellular electrophysiological recordings were carried out on dorsal raphe 5-HT neurons. The sensitivity of 5-HT_{1B} autoreceptors and α_2-adrenergic heteroreceptors, located on 5-HT terminals, and of somatodendritic 5-HT_{1D} autoreceptors, as well as that of α_2-adrenoceptors on NE terminals were also assessed.

2. Materials and methods

2.1. Animals

Male 129/SvEvTac wild-type mice and null mutant male mice lacking 5-HT_{1A} receptors, weighing 20–30 g on the day of the experiment, were used (Columbia University, Center for Neurobiology and Behavior, New York, USA). These 5-HT_{1A} mutant mice were produced from 129/SvEvTac cells and the resulting chimeras were bred with 129/SvEvTac females (Ramboz et al., 1998). Most mice used in the present experiments were imported from Columbia University where periodic genotyping was carried out. Some mice were bred at McGill University from imported and tagged pairs of mice. The animals were maintained on a 12:12-h light/dark cycle with free access to food and water. Principles established by Canadian Committee on Animal Care were followed at all times and the procedures approved by the McGill University Ethics Committee.

2.2. Extracellular unitary recordings

Mice were anesthetized with chloral hydrate (400 mg/kg, i.p. using a 4% aqueous solution) and were mounted in a stereotaxic apparatus. Additional doses (100 mg/kg, i.p.) were given to maintain the anesthesia during the experiment. The recordings were performed with single-barrelled glass micropipettes filled with 2 M NaCl. The electrodes were positionned at 0.5–0.9 mm posterior to lambda and lowered in the dorsal raphe. The neurons were usually found at a depth of 2.5–3.5 mm below the surface of the brain. They were identified using the following criteria: a gener-

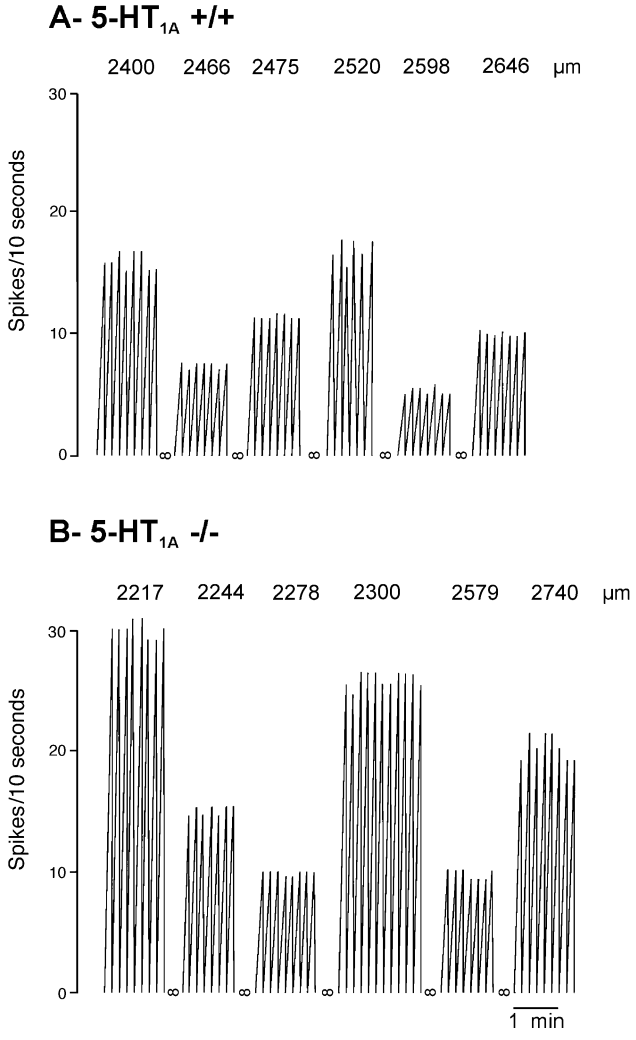

Fig. 1. Examples of representative recordings of 5-HT neurons obtained from 5-HT_{1A}+/+ and 5-$HT_{1A}-/-$ mice during systematic electrode descents through the dorsal raphe nucleus. The dots between each recording signify that the recordings were not continuous. The numbers above each tracing represent the distance between the surface of the brain and the site of recording within the dorsal raphe. The bar at the bottom of the figure applies to both traces.

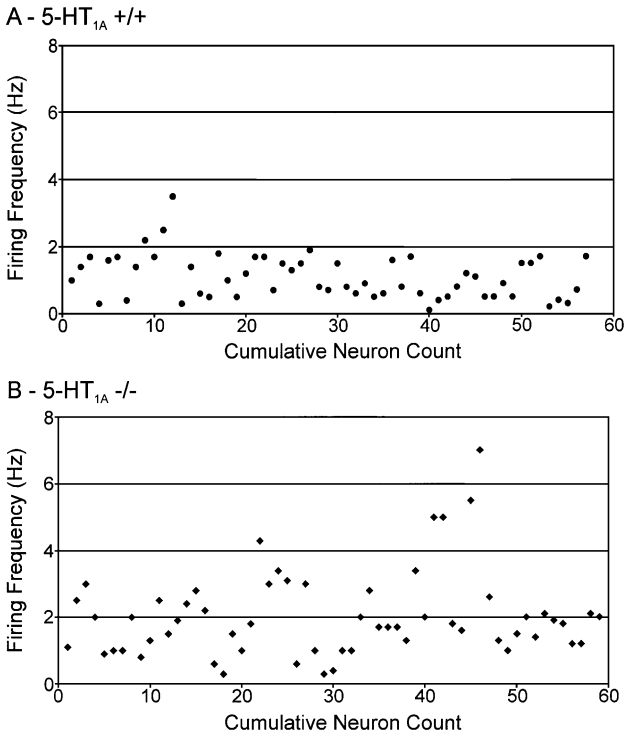

Fig. 2. Scattergrams depicting the firing frequency of all spontaneously active 5-HT neurons encountered during systematic electrode descents through the dorsal raphe of wildtype mice (A) and of 5-HT_{1A} null mutant mice (B).

ally slow (0.5 to 2.5 Hz) and regular firing rate and long-duration (0.8 to 1.2 ms) positive potentials.

2.3. Superfusion experiments

The animals were killed by decapitation and the brain immediately removed and then dissected on an ice-cold glass plate. Slices, 400-μm thick, from the mesencephalic raphe, hippocampus and frontal cortex were prepared with a McIlwain tissue chopper. The slices were then incubated for 30 min at 37 °C in Krebs buffer containing 100 nM [^{3}H]5-HT creatinine sulphate (specific activity: 21.8 Ci/mmol); NEN Research Products, MA, USA) or 100 nM [^{3}H]norepinephrine hydrochloride (specific activity: 13.5 Ci/mmol); NEN Research Products) and bubbled with a mixture of 95% O_2:5% CO_2. The composition of the Krebs solution was the following (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 1.3, $MgCl_2$ 1.2, NaH_2PO_4 1, $NaHCO_3$ 25, glucose 11.1, Na_2 EDTA 0.004 and ascorbic acid 0.11. At the end of the incubation period, the slices were washed, transferred to glass chambers, and superfused at a rate of 0.5 ml min^{-1} with oxygenated Krebs solution maintained at 37 °C. Nineteen consecutive 4-min fractions were collected starting 90 min after the beginning of superfusion for the three structures. Two periods of stimulation, S_1 and S_2, were carried out within the same experiment at 8 and 52 min, respectively, after the end of the 90-min washing period. The electrical field (30 mA, 2 ms, 3 Hz for 2 min) was generated in the chambers between two platinum electrodes positioned 2 cm apart. The frequency of stimulation was chosen because it was within the range of the firing rate of 5-HT neurons recorded in freely moving animals (Jacobs, 1986). The first stimulation period (S_1) was used as a control and the drugs were added 20 min before S_2 and remained present throughout the rest of the experiment. At the end of the superfusion period, the slices were solubilized with 0.5-ml Soluene 350 (Packard Instruments, Downers grove, IL, USA) and the radioactivity in the slices and superfusate samples was determined by liquid scintillation spectrometry. The results are expressed as the fraction of tritium content present at the time of the onset of the respective collection periods. The fractional release evoked by electrical stimulation was calculated as the difference between the total amount of radioactivity released during stimulation and the basal outflow obtained in the sample preceding the onset of stimulation (sp_1 or sp_2). To assess the drug-induced changes of electrically evoked release of tritium from the slices preloaded with [^{3}H]5-HT or [^{3}H]norepinephrine, the ratio of fractional release between the second and the first period of stimulation (S_2/S_1) were also calculated to determine whether the drugs altered the basal outflow of radioactivity. The amount of tritium released by electrical stimulation under these conditions provides a reliable estimate of the release of tritiated or endogenous 5-HT (Baumann and Waldmeier, 1981; Blier and Bouchard, 1993).

Table 1
Fraction of total tritium released from hippocampus, frontal cortex and midbrain raphe slices preloaded with [^{3}H]5-HT in 5-HT_{1A}+/+ and 5-HT_{1A} −/− mice

	Spontaneous tritium outflow (sp_1)		Electrically evoked release of [^{3}H]5-HT (S_1)	
	5-HT_{1A}+/+	5-HT_{1A} −/−	5-HT_{1A}+/+	5-HT_{1A} −/−
Hippocampus	2.23 ± 0.09 (n=67)	2.31 ± 0.11 (n=64)	1.16 ± 0.09 (n=67)	1.22 ± 0.10 (n=64)
Frontal cortex	2.04 ± 0.08 (n=60)	2.05 ± 0.07 (n=63)	1.46 ± 0.1 (n=60)	1.38 ± 0.10 (n=63)
Midbrain raphe	1.90 ± 0.10 (n=30)	2.09 ± 0.11 (n=22)	1.02 ± 0.11 (n=30)	1.06 ± 0.17 (n=22)

Spontaneous outflow of radioactivity (sp_1) refers to the percentage of total tissue radioactivity released during the 4 min preceding the first stimulation. S_1 is the fraction of total tissue radioactivity released by 360 pulses (30 mA, 2 ms, 3 Hz) in the first period of stimulation before the introduction of any drug in the perfusate. The data were generated from a total of 33, 29 and 41 mice for the three structures, respectively. None of the mean values in mutant mice were significantly different from those obtained in the wildtype mice (P>0.05) using the nonpaired Student's t test. In all experiments, slices prepared from 5-HT_{1A}+/+ and 5-HT_{1A} −/− mice (with their number given in parentheses) were processed simultaneously.

2.4. Drugs

The following drugs were used: UK14,304 (5-bromo-*N*-(4, 5-dihydro-1*H*-imidazol-2-yl)-6-quinoxalinamine; RBI, Natick, MA, USA), sumatriptan succinate (Glaxo, Middlesex, UK) and CP 93129 (1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*-pyrrol (3, 2-*b*) pyridin-5-one; Tocris, Ballwin, MO, USA).

2.5. Statistical analyses

Results are expressed as means ± S.E.M. Differences between the wildtype mice (5-HT_{1A}+/+) and the 5-HT_{1A} −/− mice were compared with the Student's two-tailed *t* test. The effects of the drugs on fractional release were assessed using one-way analyses of variance (ANOVA), with the mutant genotype as the main factor, followed when necessary by the post-hoc Tukey test. In order to detect treatment effects, the experiments were conducted by studying simultaneously in the same superfusion apparatus slices prepared from a wildtype and slices from a knockout mouse with the same drug solutions. This experimental design was deemed optimal to minimize the problem of inter-experimental variations. Probability values equal or smaller to 0.05 were considered as significant.

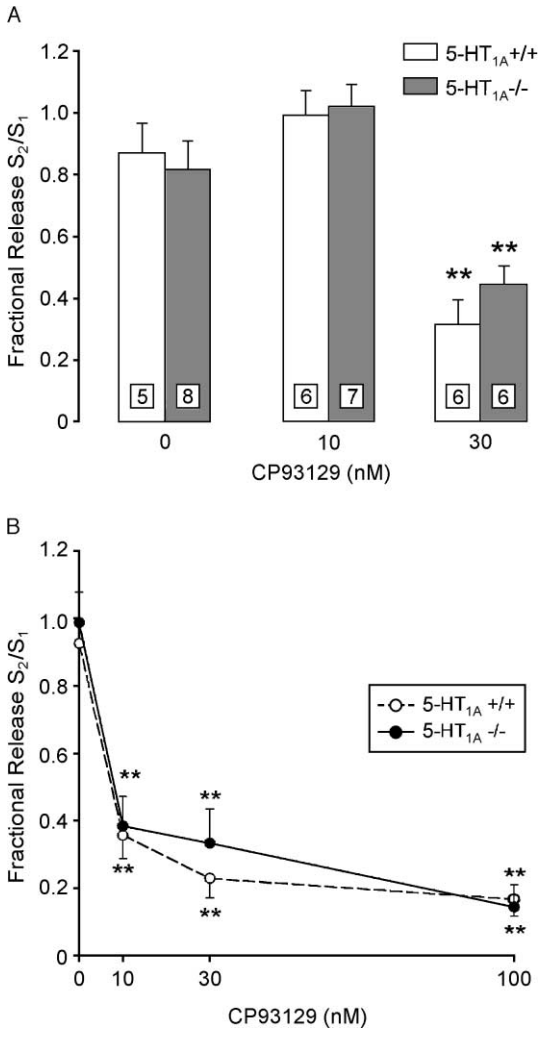

Fig. 3. Effects of the 5-HT_{1B} receptor agonist CP93129 on the inhibition of electrically evoked release of [^{3}H]5-HT from preloaded (A) frontal cortex and (B) hippocampus slices in wildtype (5-HT_{1A}+/+) and mutant (5-HT_{1A} −/−) mice. The agonist was introduced 20 min before S_2 and remained present until the end of the experiment. Values are expressed as means ± S.E.M. for at least four experiments per group of animals. * $P<0.05$; ** $P<0.01$, versus control (Tukey's test).

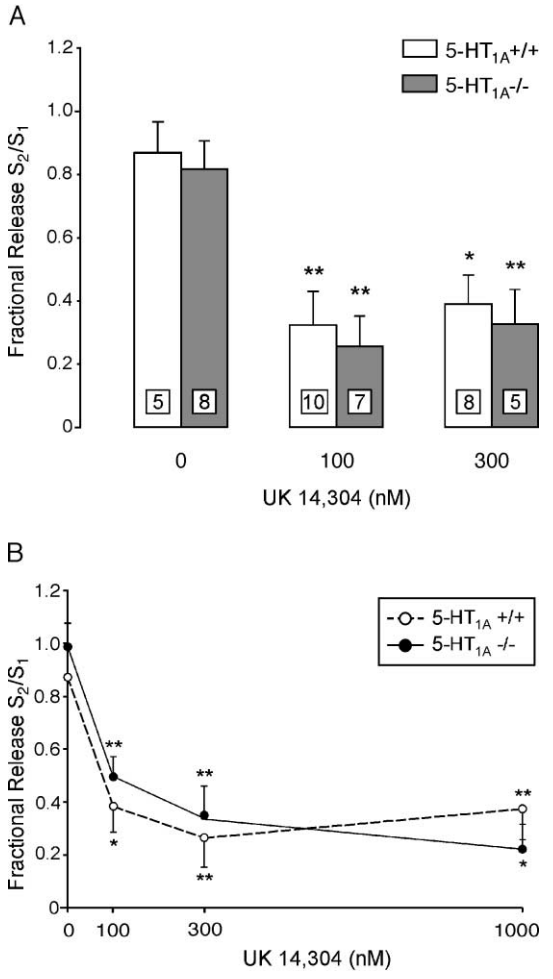

Fig. 4. Effects of the α_2-adrenoceptor agonist UK14304 on the inhibition of electrically evoked release of [^{3}H]5-HT from preloaded (A) frontal cortex and (B) hippocampus slices in wildtype (5-HT_{1A}+/+) and mutant (5-HT_{1A} −/−) mice. The agonist was introduced 20 min before S_2 and remained present until the end of the experiment. Values are expressed as means ± S.E.M. for at least four experiments per group of animals. * $P<0.05$; ** $P<0.01$, versus control (Tukey's test).

3. Results

3.1. Spontaneous firing activity of 5-HT neurons

Spontaneously active dorsal raphe 5-HT neurons were recorded from 18 5-HT_{1A}+/+ and 12 5-HT_{1A} −/− mice.

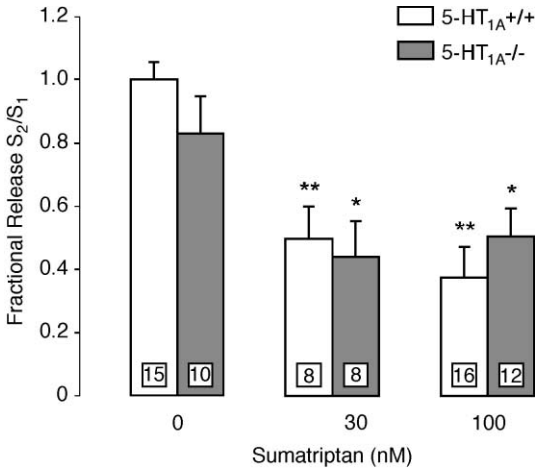

Fig. 5. Effects of the 5-$HT_{1D/1B}$ receptor agonist sumatriptan on the inhibition of electrically evoked release of [^{3}H]5-HT from preloaded mesencephalic slices in wildtype (5-HT_{1A}+/+) and mutant (5-HT_{1A} − / −) mice. The agonist was introduced 20 min before S_2 and remained present until the end of the experiment. Values are expressed as means ± S.E.M. for at least five experiments per group of animals. * $P<0.05$; * * $P<0.01$, versus control (Tukey's test).

Their mean spontaneous firing rate was nearly doubled in the 5-HT_{1A} − / − mice as compared to the 5-HT_{1A}+/+ mice (wildtype mice: 1.1 ± 0.2 Hz, $n=59$; knockout mice: 2.0 ± 0.1 Hz, $n=57$; Fig. 1). Interestingly, in the 5-HT_{1A}+/+ mice only three neurons were firing at a rate above 2 Hz, whereas in the 5-HT_{1A} − / − mice 35% of the neurons were firing in that upper range (Fig. 2).

3.2. Electrically evoked release of [^{3}H]5-HT from preloaded slices in 5-HT−/− mice

The spontaneous outflow of radioactivity (sp_1), which is mainly comprised of [^{3}H]5-hydroxyindole acetic acid, in contrast to electrically evoked tritium that is principally comprised of [^{3}H]5-HT (Blier and Bouchard, 1993), was not different in cortical, hippocampal or mesencephalic slices when comparing wildtype and mutant mice. The fractional release of [^{3}H]5-HT evoked by S_1 in the absence of any drug was also not significantly different in slices of any of these three structures from 5-HT_{1A} − / − animals as compared to the controls studied in parallel in the same experiments (Table 1). The radioactivity remaining in the slices at the end of the experiment was unchanged in the 5-HT_{1A} − / − when compared to the 5-HT_{1A}+/+ mice (data not shown).

3.3. Effect of the 5-HT_{1B} receptor agonist CP93129 on the electrically evoked release of tritium from [^{3}H]5-HT preloaded slices

The addition of CP93129 to the superfusate, 20 min before S_2, did not alter the spontaneous outflow of tritium but resulted in a significant inhibition of [^{3}H]5-HT in cortical slices [$F(2,14)=18.9$, $P<0.001$ and $F(2,18)=13.1$, $P<0.001$ for 5-HT_{1A}+/+ and 5-HT_{1A} − / − , respectively; Fig. 3A] at the 30 nM concentration (post-hoc tests, $P<0.01$ and $P<0.01$ for 5-HT_{1A}+/+ and 5-HT_{1A} − / − , respectively) that was equal in both strains of mice ($P=0.71$, $P=0.80$, $P=0.22$ for the 10 and 30 nM concentrations, respectively). In hippocampal slices, a concentration-dependent inhibition was observed [$F(3,29)=20.4$, $P<0.001$ and $F(3,27)=12.6$, $P<0.001$ for 5-HT_{1A}+/+ and 5-HT_{1A} − / − , respectively; Fig. 3B] for 10 (post-hoc tests, $P<0.01$ and $P<0.01$ for 5-HT_{1A}+/+ and 5-HT_{1A} − / − , respectively), 30 (post-hoc test, $P<0.01$ and $P<0.01$ for 5-HT_{1A}+/+ and 5-HT_{1A} − / − , respectively) and 100 nM concentrations (post-hoc tests, $P<0.01$ and $P<0.01$ for 5-HT_{1A}+/+ and 5-HT_{1A} − / − , respectively). However, the degree of inhibition was equal in both strains of mice ($P=0.43$, $P=0.80$, $P=0.48$, $P=0.79$ for the 0, 10, 30 and 100 nM concentrations, respectively).

3.4. Effect of the α_2-adrenoceptor agonist UK14,304 on the electrically evoked release of tritium from [^{3}H]5-HT preloaded slices

The fractional release of [^{3}H]5-HT evoked in S_2 was significantly diminished [$F(2,20)=6.4$, $P<0.01$ and $F(2,21)=9.1$, $P<0.001$ for 5-HT_{1A}+/+ and 5-HT_{1A} − / − , respectively; Fig. 4A] by the addition of 100 (post-hoc test, $P<0.01$ and $P<0.01$ for 5-HT_{1A}+/+ and 5-HT_{1A} − / − , respectively) and 300 nM of the α_2-adrenoceptor agonist UK14,304 in the cortex (post-hoc tests, $P<0.05$ and $P<0.01$ for 5-HT_{1A}+/+ and 5-HT_{1A} − / − , respectively), as previously reported for

Table 2
Fraction of total tissue tritium released from hippocampus and frontal cortex slices preloaded with [^{3}H]NE in 5-HT_{1A}+/+ and 5-HT_{1A} − / − mice

	Spontaneous tritium outflow (sp_1)		Electrically evoked release of [^{3}H]NE (S_1)	
	5-HT_{1A}+/+	5-HT_{1A} − / −	5-HT_{1A}+/+	5-HT_{1A} − / −
Hippocampus	0.80 ± 0.1 ($n=19$)	0.74 ± 0.09 ($n=18$)	1.93 ± 0.30 ($n=19$)	1.57 ± 0.22 ($n=18$)
Frontal cortex	0.80 ± 0.07 ($n=17$)	0.81 ± 0.07 ($n=16$)	1.55 ± 0.13 ($n=17$)	1.68 ± 0.14 ($n=16$)

Spontaneous outflow of radioactivity (sp_1) refers to the percentage of total tissue radioactivity released during the 4 min preceding the first stimulation. S_1 is the fraction of total tissue radioactivity released by 360 pulses (30 mA, 2 ms, 3 Hz) in the first period of stimulation before the introduction of any drug in the perfusate. The data were generated from a total of 12 mice for each structure. None of the mean values in mutant mice were significantly different from those obtained in the wildtype mice ($P>0.05$) using the nonpaired Student's t test. In all experiments, slices prepared from 5-HT_{1A}+/+ and 5-HT_{1A} − / − mice were processed simultaneously.

other species (Blier et al., 1990; Blier and Bouchard, 1994). However, no significant change was observed between 5-HT_{1A}+/+ and 5-HT_{1A} −/− animals ($P=0.71$, $P=0.66$ and 0.67 for the 0, 100 and 300 nM concentrations, respectively). In the hippocampus, a significant inhibition was also observed [one-way ANOVAs, $F(3,30)=6.5$, $P<0.002$ and $F(3,27)=8.0$, $p<0.001$ for 5-HT_{1A}+/+ and 5-HT_{1A} −/−, respectively; Fig. 4B] after the addition of 100 (post-hoc tests, $P<0.01$ and $P<0.05$ for 5-HT_{1A}+/+ and 5-HT_{1A} −/−, respectively), 300 (post-hoc tests, $P<0.01$ and $P<0.01$ for 5-HT_{1A}+/+ and 5-HT_{1A} −/−, respectively) and 1000 nM (post-hoc tests, $P<0.05$ and $P<0.01$ for 5-HT_{1A}+/+ and 5-HT_{1A} −/−, respectively), but no difference resulted between the two strains of mice ($P=0.43$, $P=0.34$, $P=0.37$ and $P=0.33$ for the 0, 100, 300 and 1000 nmol/l concentrations, respectively).

3.5. Effect of the 5-$HT_{1D/1B}$ receptor agonist sumatriptan on the electrically evoked release of tritium from [^{3}H]5-HT preloaded slices

Sumatriptan produced a significant diminution in the release of [^{3}H]5-HT by preloaded mesencephalic slices [$F(2,36)=16.7$, $P<0.001$ and $F(2,27)=3.8$, $P<0.03$ for 5-HT_{1A}+/+ and 5-HT_{1A} −/−, respectively; Fig. 5] at the 30 (post-hoc tests, $P<0.01$ and $P<0.05$ for 5-HT_{1A}+/+ and 5-HT_{1A} −/−, respectively) and 100 nM concentrations (post-hoc tests, $P<0.01$ and $P<0.05$ for 5-HT_{1A}+/+ and 5-HT_{1A} −/−, respectively), without altering the spontaneous outflow of tritium. No difference was observed in the 5-HT_{1A} −/− when compared to the 5-HT_{1A}+/+ mice ($P=0.16$, $P=0.72$ and $P=0.35$ for the 0, 30 and 100 nM concentrations, respectively).

3.6. Effect of the α_2-adrenoceptor agonist UK14,304 on the electrically evoked release of tritium from [^{3}H]NE preloaded slices

In control conditions, the spontaneous outflow of radioactivity (sp_1) was not significantly different, neither in cortical nor in hippocampal slices preloaded with [^{3}H]norepinephrine in 5-HT_{1A} −/− and 5-HT_{1A}+/+ mice (Table 2). The electrically evoked release of [^{3}H]norepinephrine in S_1 was also unaffected in mutant animals when compared to that of normal littermates in both structures (Table 2). The release of [^{3}H]norepinephrine was decreased in preloaded cortical slices from knockout and control animals following the addition of UK14,304 to the superfusate [$F(2,12)=28.7$, $P<0.001$ and $F(2,11)=57.3$, $P<0.001$ for 5-HT_{1A}+/+ and 5-HT_{1A} −/−, respectively; Fig. 6A] at the 10 (post-hoc tests, $P<0.01$ and $P<0.01$ for 5-HT_{1A}+/+ and 5-HT_{1A} −/−, respectively) and 100 nM concentrations (post-hoc tests, $P<0.01$ and $P<0.01$ for 5-HT_{1A}+/+ and 5-HT_{1A} −/−, respectively). However, these concentrations produced an equal inhibition in both groups of mice ($P=0.85$, $P=0.44$ and $P=0.53$ for the 0, 10 and 100 nM concentrations, respectively). In hippocampal slices, UK14,304 inhibited the evoked release of [^{3}H]norepinephrine from both strains [$F(2,13)=14.3$, $P<0.001$ and $F(2,13)=7.9$, $P<0.01$ for 5-HT_{1A}+/+ and 5-HT_{1A} −/−, respectively; post-hoc tests for 10 nM: $P<0.01$ and $P<0.05$ for 5-HT_{1A}+/+ and 5-HT_{1A} −/−, respectively; post-hoc tests for 100 nM: $P<0.01$ and $P<0.01$ for 5-HT_{1A}+/+ and 5-HT_{1A} −/−, respectively; Fig. 6B]. These concentrations of the α_2-adrenoceptor agonist produced the same degree of inhibition in both groups of mice ($P=0.36$, $P=0.44$ and 0.57 for the 0, 10 and 100 nM concentrations, respectively).

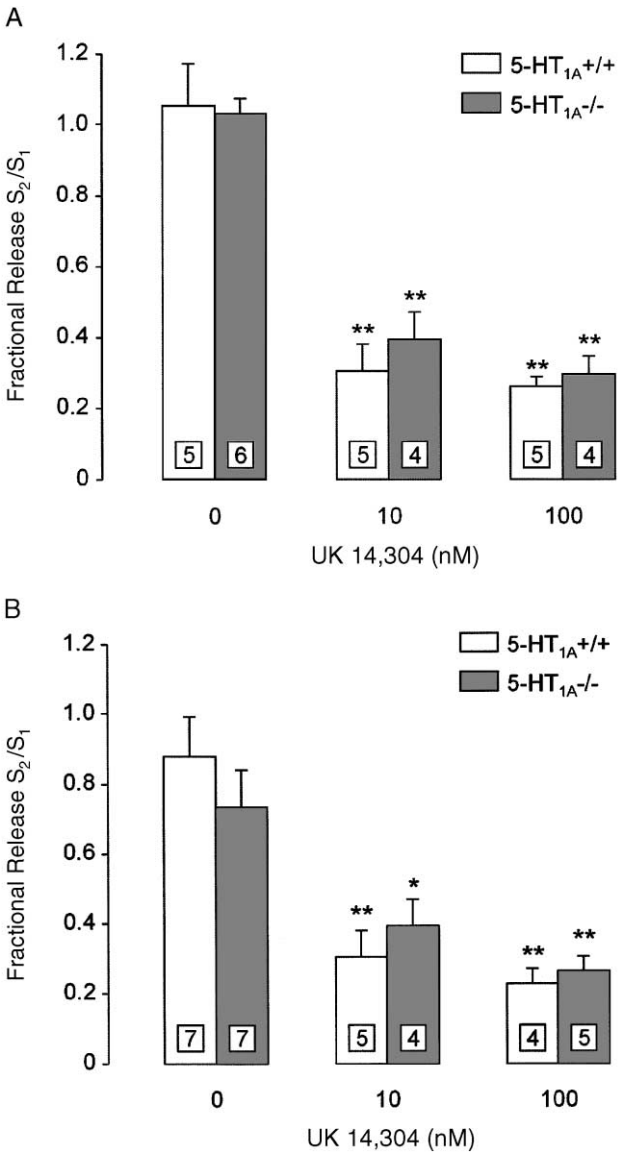

Fig. 6. Effects of the α_2-adrenoceptor agonist UK14304 on the inhibition of electrically evoked release of [^{3}H]norepinephrine from preloaded (A) frontal cortex and (B) hippocampus slices in wildtype (5-HT_{1A}+/+) and mutant (5-HT_{1A} −/−) mice. The agonist was introduced 20 min before S_2 and remained present until the end of the experiment. Values are expressed as means ± S.E.M. for at least four experiments per group of animals. * $P<0.05$; ** $P<0.01$, versus control (Tukey's test).

4. Discussion

The results of the present study indicate that the firing rate of dorsal raphe 5-HT neurons was markedly increased in 5-HT_{1A} −/− mice because of the absence of the 5-HT_{1A} autoreceptors (Ramboz et al., 1998; see Figs. 1 and 2). In contrast, the electrically evoked release of [^{3}H]5-HT from preloaded brain slices was unaltered in 5-HT_{1A} −/− mice, as well as its modulation by 5-HT_{1D}, 5-HT_{1B} and α_2-adrenergic receptors in various brain structures. In addition, the genetic deletion of the 5-HT_{1A} receptor did not result in a modification of the electrically evoked release of [^{3}H]norepinephrine from preloaded brain slices or in an altered capacity of α_2-adrenergic autoreceptors to diminish norepinephrine release in two forebrain structures.

Considering that 5-HT_{1A} receptors mediate an inhibitory effect on the firing activity of 5-HT neurons, an increase in firing rate in 5-HT_{1A} −/− mice was expected. Indeed, the mean spontaneous firing rate of dorsal raphe 5-HT neurons was increased by 90% in 5-HT_{1A} −/− animals as compared to their wildtype littermates (Figs. 1 and 2). Similarly, in neurokinin 1 null mutant mice, the spontaneous firing rate of 5-HT neurons is enhanced to the same degree as reported in the present study in 5-HT_{1A} −/− mice (Santarelli et al., 2001). The latter phenomenon was attributed to a marked desensitization of the 5-HT_{1A} autoreceptor in these mice lacking the receptor on which substance P acts. Taken together, these results indicate that when a loss of the inhibitory effect of the 5-HT_{1A} autoreceptor is achieved, without interfering with other inactivating mechanisms of 5-HT, the firing rate of 5-HT neurons is approximately doubled, when these neurons are recorded under anesthesia. Consistent with the present observations, Ase et al. (2000) have recently observed an increased 5-HT turnover, as measured by the determination of the 5-HT/5-HIAA ratio, in the dorsal/median raphe. However, these authors concluded this alteration should result in an increased release of 5-HT at the cell body level of 5-HT neurons, which was actually not seen in the present experiments (Table 1). Nevertheless, it is important to mention that the rate of neuronal depolarization in the present study was driven at a fixed rate of 3 Hz. Consequently, only microdialysis experiments carried out under basal conditions will determine whether 5-HT release in the raphe area is enhanced in 5-HT_{1A} −/− mice. It is quite possible that this parameter would not be increased because about 65% of 5-HT neurons in 5-HT_{1A} −/− mice were still firing in the range of those in the 5-HT_{1A}+/+ mice (Fig. 2).

The brain slice superfusion paradigm used in the present study has been used in the past to document changes in receptor sensitivity following various pharmacological treatments. For instance, it has been shown by two groups of investigators that a chronic treatment with selective serotonin reuptake inhibitors enhances 5-HT release and desensitizes terminal 5-HT_{1B} autoreceptors (Blier and Bouchard, 1994; El Mansari et al., 1995; Moret and Briley, 1990), consistent with results obtained in vivo electrophysiological studies (Chaput et al., 1986, 1991). Moreover, it has been reported that long-term administration of monoamine oxidase and of norepinephrine reuptake inhibitors diminish the function of α_2-adrenoceptors controlling the release of 5-HT in the hippocampus, using both experimental approaches (see Mongeau et al., 1997 for a review). Thus, given the similar release of 5-HT obtained herein in normal and mutant mice, it may be concluded that the absence of 5-HT_{1A} receptors did not lead to any alteration of the responsiveness of the presynaptic receptors that were studied. Preliminary data obtained using only the IC_{50} concentration of the 5-HT_{1B} receptor agonist CP93129 to study the sensitivity of the 5-HT_{1B} autoreceptor in the hippocampus suggested that the latter receptor was sensitized in 5-HT_{1A} −/− mice (Ramboz et al., 1998). Clearly, upon studying more than one concentration of this 5-HT_{1B} receptor agonist, the present results did not confirm this initial observation (Fig. 3B). Results from microdialysis experiments examining the decrease of extracellular levels of 5-HT by local perfusion of the same 5-HT_{1B} receptor agonist are fully consistent with the results presented herein (Knobelman et al., 2001). Furthermore, it is important to mention that both the present superfusion paradigm and the microdialysis approach have revealed that 5-HT release is unaltered in 5-HT_{1B} −/− mutant mice, despite the loss of the negative autoregulatory influence of the terminal 5-HT_{1B} autoreceptor (Pineyro et al., 1995; Trillat et al., 1997; Knobelman et al., 2001).

There are now three microdialysis studies that examined the extracellular levels of 5-HT in postsynaptic structures of 5-HT_{1A} −/− mutant mice. Two showed unaltered levels of 5-HT in the striatum and/or the hippocampus (He et al., 2001; Knobelman et al., 2001) and one an increase in the frontal cortex and the hippocampus (Parsons et al., 2001). It is always possible to claim that such divergent results could be attributed to the use of different background strains of mice, but the one striking difference of the latter work, showing an altered level of 5-HT, is that much older mice were used. It is important to emphasize here that the present experiments were carried out with mice originating from the same laboratory and were approximately of the same age as those used by Knobelman et al. (2001). Both studies yielded identical results with respect to 5-HT levels and 5-HT_{1B} autoreceptor sensitivity.

One possibility to envisage for the lack of alteration of 5-HT release in the 5-HT_{1A} −/− mice is that the 5-HT transporter could theoretically be up-regulated to compensate for the increased number 5-HT molecules released into synaptic cleft. Nevertheless, the present results would not support this contention as the amount of [^{3}H]5-HT remaining in the slices after the experiments, a reliable index of reuptake in the presence of unaltered release and presynaptic receptor function, was not different in the 5-HT_{1A} −/− mice when compared to the controls. This conclusion is also in line with previous immunohistochemical results showing a normal density and distribution of the 5-HT transporter in these animals (Heisler et al., 1998).

The noradrenergic and 5-HT systems are known to interact extensively (Mongeau et al., 1997). For instance, although subacute administration of selective serotonin reuptake inhibitors does not alter the firing rate of locus coeruleus noradrenergic neurons, a robust inhibitory effect gradually develops as the treatment is prolonged (Szabo et al., 1999, 2000). Moreover, many pharmacological compounds targeting primarily the noradrenergic system, like the selective norepinephrine reuptake inhibitors desipramine, nisoxetine and reboxetine, also induce profound alterations of 5-HT neuronal elements after long-term administration (Mongeau et al., 1997; Szabo and Blier, 2001; Yoshioka et al., 1995). It therefore remains possible that, even if no change in noradrenergic neuronal function has been identified in the present study, potential mutation-induced alterations in noradrenergic neuronal function could have been dampened by the 5-HT system. For instance, it will be interesting to examine the firing rate of noradrenergic neurons in 5-HT_{1A} −/− mice, given that 5-HT neurons exert a tonic inhibitory action on the latter parameter and that 5-HT turnover is increased in the locus coeruleus of 5-HT_{1A} −/− mice (Ase et al., 2000; Haddjeri et al., 1997b).

In conclusion, the present study documented a marked increase in the mean firing activity of 5-HT neurons. However, it did not put into evidence alterations of the electrically evoked release of [^{3}H]5-HT and [^{3}H]norepinephrine in 5-HT_{1A} −/− mice nor of the presence of adaptive changes of the responsiveness of presynaptic 5-HT and noradrenergic receptors controlling the release of these two neurotransmitters. Since the firing rate of 5-HT neurons was nearly doubled in these mice, it will be interesting to determine which 5-HT neuronal elements allow such an apparent homeostasis to be maintained. Finally, based on the present results, it is possible to tentatively conclude that the anxious phenotype of 5-HT_{1A} −/− mice is mainly due to the absence of the postsynaptic 5-HT_{1A} receptors because 5-HT release appears to be normal in the mutant mice. Therefore, there should not be any compensation occurring by altered 5-HT release at 5-HT receptor subtypes other than the 5-HT_{1A} subtype in 5-HT_{1A} −/− mice.

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