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In vivo efflux of serotonin in the dorsal raphe nucleus of 5-HT_{1A} receptor knockout mice

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Abstract

In the dorsal raphe nucleus (DR), extracellular serotonin (5-HT) regulates serotonergic transmission through 5-HT_{1A} autoreceptors. In this work we used *in vivo* microdialysis to examine the effects of stressful and pharmacological challenges on DR 5-HT efflux in 5-HT_{1A} receptor knockout (5-HT_{1A}–/–) mice and their wild-type counterparts (5-HT_{1A}+/+). Baseline 5-HT concentrations did not differ between both lines of mice, which is consistent with a lack of tonic control of 5-HT_{1A} autoreceptors on DR 5-HT release. (*R*)-(+)-8-Hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT, 0.5 mg/kg) reduced 5-HT levels to 30% of basal values in 5-HT_{1A}+/+ mice, but not in 5-HT_{1A}–/– mice. The selective 5-HT_{1B} receptor agonist 1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*-pyrrolo[3,2-b]pyridin-5-one dihydrochloride (CP 93129, 300 μM) reduced dialysate 5-HT to the same extent (30–40% of baseline) in the two genotypes, which suggests a lack of compensatory changes in 5-HT_{1B} receptors in the DR of such mutant mice. Both a saline injection and handling for 3 min increased DR dialysate 5-HT in mutants, but not in 5-HT_{1A}+/+ mice. Fluoxetine (5 and 20 mg/kg) elevated 5-HT in a dose-dependent manner in both genotypes. However, this effect was markedly more pronounced in the 5-HT_{1A}–/– mice. The increased responsiveness of the extracellular 5-HT in the DR of 5-HT_{1A} receptor knockout mice reflects a lack of the autoinhibitory control exerted by 5-HT_{1A} autoreceptors.

Keywords: dorsal raphe nucleus, fluoxetine, 5-HT_{1A} receptor knockout mice, 5-HT_{1B} receptors, microdialysis, stress.
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The serotonin (5-hydroxytryptamine [5-HT]) system participates in many physiological functions (Jacobs and Azmitia 1992) and is involved in several psychiatric disorders (Caldecott-Hazard *et al*. 1991; Mann 1999). The multiple functions of the transmitter have been attributed to its action on 14 different subtypes of 5-HT receptors recognized so far (Barnes and Sharp 1999). Among them, 5-HT_{1A} receptors play a crucial role in regulating the activity of 5-HT neurons. In the mammalian brain, the principal source of the serotonergic innervation of the forebrain is the dorsal raphe nucleus (DR) located in the midbrain (Azmitia and Segal 1978; Imai *et al*. 1986; Jacobs and Azmitia 1992). Within the DR, 5-HT_{1A} receptors act as autoreceptors controlling 5-HT cell firing (Sprouse and Aghajanian 1986; VanderMaelen *et al*. 1986; Sinton and Fallon 1988) and release (Hutson *et al*. 1989; Sharp *et al*. 1989; Bonvento *et al*. 1992; Adell *et al*. 1993; Kreiss and Lucki 1994). However, 5-HT_{1A} autoreceptors do not appear to be tonically activated by the endogenous transmitter (Adell *et al*. 2002; Johnson *et al*. 2002). Therefore, it could be expected that mice lacking this receptor, although being devoid of such an autoinhibitory control, would exhibit an unchanged basal 5-HT efflux. In line with this hypothesis, it has been observed that the spontaneous firing rate of DR serotonergic neurons in 5-HT_{1A} receptor knockout mice is in the same range as their wild-type counterparts (Richer *et al*. 2002; Adell *et al*. 2003), although in a subpopulation of such neurons the discharge rate nearly doubled (Richer *et al*. 2002). In addition, the genetic disruption of 5-HT_{1A} receptors does not appear to alter either the *in vivo* efflux of 5-HT in forebrain regions

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Abbreviations used: CP 93129, 1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*-pyrrolo[3,2-b]pyridin-5-one dihydrochloride; DR, dorsal raphe nucleus; 5-HT, 5-hydroxytryptamine or serotonin; 8-OH-DPAT, (*R*)-(+)-8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide; SSRI, selective serotonin reuptake inhibitor.

(He *et al*. 2001; Knobelman *et al*. 2001b; Adell *et al*. 2003) or the *in vitro* release of [^{3}H]5-HT from slices of the forebrain or the midbrain containing the raphe nuclei (Ramboz *et al*. 1998; Richer *et al*. 2002). Nevertheless, a recent study reported increased dialysate 5-HT in the frontal cortex and hippocampus of 8–10-month-old 5-HT$_{1A}$ receptor knockout mice (Parsons *et al*. 2001). It is possible that age and/or genetic background, as well as environmental conditions are responsible for these discrepancies. In contrast to the above hypothesis, an increased total tissue content of the 5-HT metabolite 5-hydroxyindoleacetic acid has been found in several brain regions of 5-HT$_{1A}$ receptor knockout mice, including the DR (Ase *et al*. 2000). However, this finding is not consistent with that of Ramboz *et al*. (1998), although both studies agree in that the concentration of 5-HT throughout the brain is not altered in mice lacking 5-HT$_{1A}$ receptors.

The presence of 5-HT in the extracellular space within the raphe nuclei can regulate the release of the transmitter throughout the brain by means of the activation of somatodendritic 5-HT$_{1A}$ receptors (for review, see Adell *et al*. 2002). However, in spite of the importance of extracellular 5-HT in the raphe nuclei, to our knowledge no study has been conducted *in vivo* to determine possible differences in the efflux of 5-HT in the DR of 5-HT$_{1A}$ receptor knockout mice. For this reason, in the present work, we have examined the effects of stress and the selective serotonin reuptake inhibitor (SSRI) fluoxetine on the *in vivo* efflux of 5-HT in the DR of mutant mice and their wild-type counterparts. In addition, the responsiveness of local 5-HT$_{1B}$ receptors was also studied to determine whether adaptive changes might have developed as a consequence of the constitutive inactivation of the gene coding for the 5-HT$_{1A}$ receptor.

Materials and methods

Animals

Male homozygous 5-HT$_{1A}$ receptor knockout (5-HT$_{1A}$–/–) and wild-type (5-HT$_{1A}$+/+) mice had the same genetic background, i.e. C57BL/6, and were 10–12 weeks old. 5-HT$_{1A}$ receptor knockout mice were generated at Princeton University by homologous recombination, as previously described (Parks *et al*. 1998). From this initial source some individuals were transferred in order to grow a stable colony in the animal facility of the University of Barcelona School of Medicine. The animals were maintained on a 12-h light/dark cycle (lights on at 07.00 h) and housed four to six per cage before surgery and individually after surgery. Food and water were always freely available. All experimental procedures were carried out in strict accordance with European Communities Council Directive on 'Protection of Animals Used in Experimental and Other Scientific Purposes' of 24 November 1986 (86/609/EEC) and were approved by the Institutional Animal Care and Use Committees. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Chemicals and drugs

All the reagents were of analytical grade and obtained from Merck (Darmstadt, Germany). Fluoxetine hydrochloride, (*R*)-(+)-8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT), 5-HT oxalate, neutral red, fast green and formalin solution 10% neutral buffered were purchased from Sigma (Saint Louis, MO, USA), and 1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*-pyrrolo[3,2-b]pyridin-5-one dihydrochloride (CP 93129) from Tocris (Bristol, UK). Citalopram hydrobromide was generously donated by H. Lundbeck A/S (Copenhagen-Valby, Denmark). Fluoxetine and 8-OH-DPAT were dissolved in distilled water and saline, respectively, and injected intraperitoneally (i.p.) in a volume of 2 mL/kg. CP 93129 was dissolved in artificial CSF. All doses and concentrations are referred as free base. Groups of mice were perfused with artificial CSF and injected with distilled water or saline to serve as control for the systemic administration of drugs.

Dialysis procedures

Concentric dialysis probes were constructed as previously described (Bortolozzi *et al*. 2003). Briefly, the shaft of the probe was made up of 15-mm long, 25 gauge (0.51 mm OD, 0.30 mm ID) stainless-steel tubing (A-M Systems, Carlsborg, WA, USA). The inflow and outflow tubes threaded through the 25 gauge tubing consisted of fused silica capillary tubing of 0.11 mm OD and 0.04 mm ID (Composite Metal Services Ltd, The Chase, Hallow, UK). The upper exposed ends of silica tubings were inserted into 7-mm long, 27 gauge (0.41 mm OD, 0.20 mm ID) stainless-steel tubing. The probes were secured to the skull with dental cement and two 2-mm long, 0.95-mm diameter screws (Microbiotech/se AB, Stockholm, Sweden). Mice were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and mounted in a stereotaxic frame (David Kopf, Tujunga, CA, USA). Each mouse was implanted with one dialysis probe equipped with a Cuprophan membrane (1-mm long; 5000 Da molecular weight cut-off) in the DR. Stereotaxic coordinates (in mm) were AP −4.5, L −1.0, DV −4.4, with a lateral angle of 20°, from bregma and top of the skull according to Franklin and Paxinos (1997). Microdialysis experiments were conducted 20–24 h after surgery in freely moving mice by continuously perfusing probes with artificial CSF containing 125 mM NaCl, 2.5 mM KCl, 1.26 mM $CaCl_2$ and 1.18 mM $MgCl_2$. In some experiments, 1 μM citalopram was added to the artificial CSF. When the effects of fluoxetine on 5-HT efflux were examined, citalopram was omitted from the perfusion fluid to avoid confounding effects between both selective serotonin reuptake inhibitors. The artificial CSF was perfused at a rate of 1.5 μL/min with a WPI model sp220i syringe pump (WPI, Aston, Stevenage, UK) attached to an overhead liquid swivel (Instech, Plymouth Meeting, PA, USA). Dialysate samples of 30 μL were collected every 20 min in microcentrifuge vials. After a 100-min stabilization period, four dialysate samples were collected to obtain basal 5-HT values before pharmacological treatment (8-OH-DPAT, CP 93129 or fluoxetine) or behavioral manipulation (saline injection or handling for 3 min). At the completion of dialysis experiments, mice were given an overdose of sodium pentobarbital and a fast green solution was perfused through the dialysis probes to stain the surrounding tissue. Then the animals were perfused transcardially with 0.9% saline followed by 10% buffered formalin. Each brain was removed immediately, frozen at

−70°C and cut afterwards on a cryostat in the coronal plane at 50 μm. Each of the sections was then stained with neutral red, according to standard procedures, for localization of the site of perfusion.

Biochemical determinations

The concentration of 5-HT in dialysate samples was determined by a HPLC method described previously (Adell and Artigas 1998). In short, 5-HT was separated on a 3-μm octadecylsilica column (7.5 cm × 0.46 cm; Beckman, San Ramon, CA, USA) and detected amperometrically with a Hewlett-Packard 1049 detector (Palo Alto, CA, USA) set at an oxidation potential of 0.6 V. The detection limit for 5-HT was estimated to be around 1 fmol/sample. Quantification of 5-HT was carried out by an external standard method using a Nelson Turbochrom Navigator (Perkin-Elmer, San Jose, CA, USA).

Data analysis

The content of 5-HT in each sample was expressed as percentage of the average baseline level calculated from four fractions collected before treatment. Data correspond to mean ± SEM values of the percentage obtained in each experimental group. The changes in dialysate 5-HT were analyzed by two-way repeated measures analysis of variance (ANOVA). When significant effects were found, post-hoc comparisons were made with Tukey's HSD multiple comparison test. The regional differences in basal dialysate concentrations of 5-HT between both strains were assessed by Student's *t*-test (two-tailed). The level of significance was set at $p < 0.05$. All statistical procedures were performed using the Statistica software for Windows (StatSoft, Tulsa, OK, USA).

Results

Baseline 5-HT values

A representative site of a dialysis probe placement is shown in Fig. 1. In the absence of citalopram in the perfusion fluid, the basal values of 5-HT in dialysate samples of the DR were 6.1 ± 0.8 fmol/30 μL ($n = 18$) in 5-HT$_{1A}$+/+ and 5.9 ± 0.5 fmol/30 μL ($n = 33$) in 5-HT$_{1A}$–/– mice. In the presence of citalopram, the corresponding basal values were 46.4 ± 8.9 fmol/30 μL ($n = 17$) in 5-HT$_{1A}$+/+ and 54.7 ± 7.9 fmol/30 μL ($n = 29$) in 5-HT$_{1A}$–/– mice. The inclusion of citalopram in the perfusion fluid elevated dialysate 5-HT in the DR of wild-type and knockout mice to a comparable extent (approximately eightfold). Both in the presence or absence of citalopram in the artificial CSF, there was no significant difference in the basal 5-HT values between the two lines of mice.

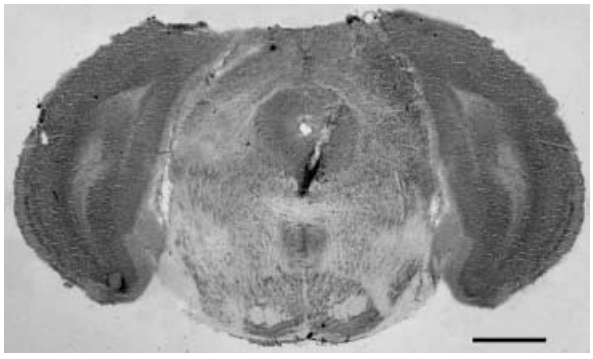

Fig. 1 Representative histological section cut in the coronal plane at 50 μm showing the tract of a dialysis probe located within the dorsal raphe nucleus of the mouse. Scale bar, 1 mm.

Effects of 8-OH-DPAT

This experiment was conducted in the presence of 1 μM citalopram in the perfusion fluid to allow comparison with previous data. At this low concentration, local infusion of citalopram does not result in substantial activation of autoreceptors (Adell *et al.* 1993; Matos *et al.* 1996). As depicted in Fig. 2 (upper panel), the systemic administration of 0.5 mg/kg 8-OH-DPAT to 5-HT$_{1A}$+/+ mice reduced DR

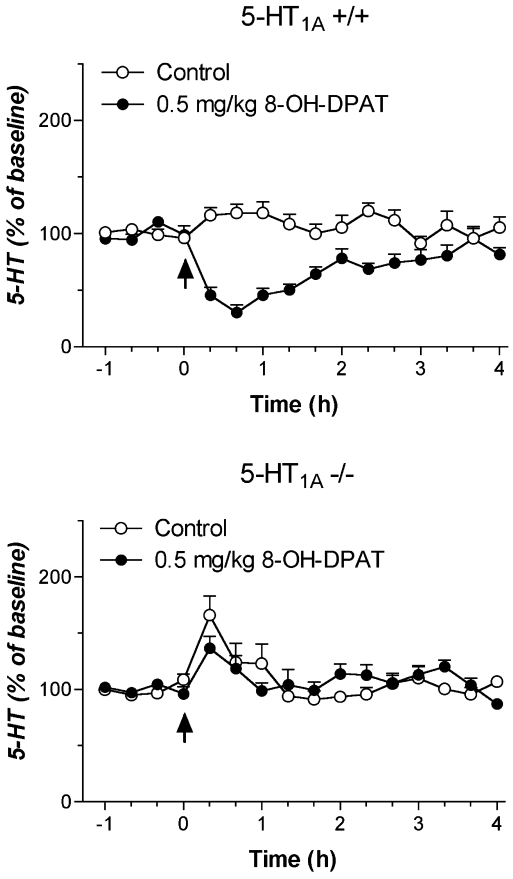

Fig. 2 Effects of the injection (arrows) of saline (control) or 0.5 mg/kg 8-OH-DPAT on dialysate 5-HT in the DR of 5-HT$_{1A}$+/+ mice (top panel) and 5-HT$_{1A}$–/– mice (bottom panel). In 5-HT$_{1A}$+/+ mice, a saline injection ($n = 5$) had no effect, whereas 8-OH-DPAT ($n = 5$) significantly decreased dialysate 5-HT ($p < 0.0002$). In 5-HT$_{1A}$–/– mice, a transient increase in dialysate 5-HT ($p < 0.05$, Tukey's HSD test) was observed following the injection of saline ($n = 8$) or 8-OH-DPAT ($n = 7$).

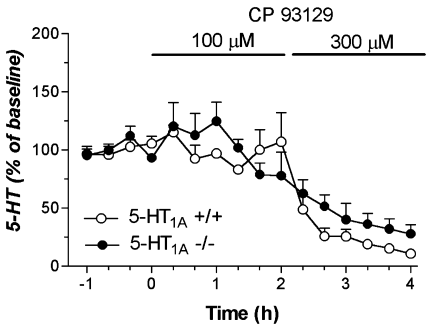

Fig. 3 Effects of the local perfusion of CP 93129 on dialysate 5-HT in the DR of 5-HT$_{1A}$+/+ mice ($n = 4$) and 5-HT$_{1A}$–/– mice ($n = 5$). The concentration of 100 μM CP 93129 failed to alter dialysate 5-HT, but that of 300 μM reduced dialysate 5-HT to a comparable extent in both genotypes ($p < 0.05$, Tukey's HSD test).

dialysate 5-HT to 30% of basal values ($p < 0.0002$), but this effect was completely abolished in 5-HT$_{1A}$ receptor knockout mice (Fig. 2, lower panel). However, DR dialysate 5-HT was increased in 5-HT$_{1A}$–/– mice as a consequence of the injection procedure, regardless of the solution injected (saline or 8-OH-DPAT). This is shown by the significant effect of time ($p < 0.001$), but not of treatment ($p = 0.91$) or the interaction between both factors ($p = 0.07$).

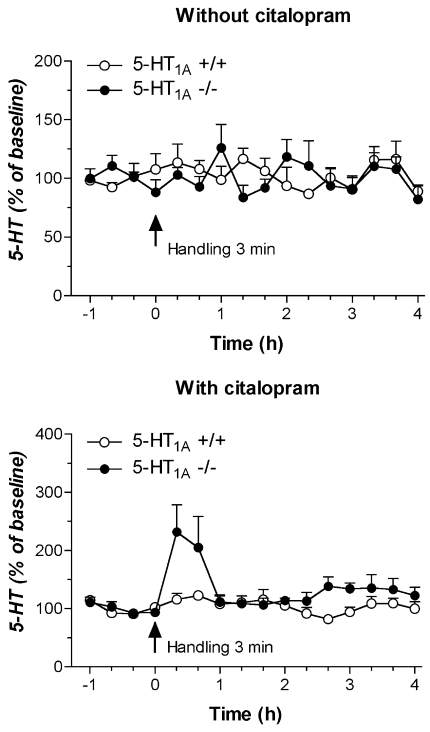

Fig. 4 Effects of handling for 3 min (arrow) on dialysate 5-HT in the DR of 5-HT$_{1A}$+/+ and 5-HT$_{1A}$–/– mice. In the absence of citalopram in the perfusion fluid (upper panel), handling produced no effect in either 5-HT$_{1A}$+/+ mice ($n = 4$) or 5-HT$_{1A}$–/– mice ($n = 5$). In the presence of 1 μM citalopram in the perfusion fluid (lower panel), handling produced no effect in 5-HT$_{1A}$+/+ mice ($n = 5$), but a significant increase in dialysate 5-HT ($p < 0.05$, Tukey's HSD test) was observed at 20 and 40 min in 5-HT$_{1A}$–/– mice ($n = 5$).

Effects of CP 93129

In this experiment, 1 μM citalopram was also added to the perfusion fluid to allow comparison with previous data on the effects of CP 93129 on 5-HT efflux in the DR of rats (Adell *et al*. 2001). The results are depicted in Fig. 3. ANOVA revealed significant effect of time ($p < 0.00001$), but not of genotype ($p = 0.67$) or the interaction between both factors ($p = 0.15$). Post-hoc comparisons showed that 100 μM CP 93129 was without effect. The effect of 300 μM CP 93129 in reducing dialysate 5-HT was apparently more marked in wild-type mice, but the difference between both genotypes did not reach statistical significance.

Effects of handling

In the present work, handling was defined as picking a mouse up and holding it tightly in the hand of the experimenter for

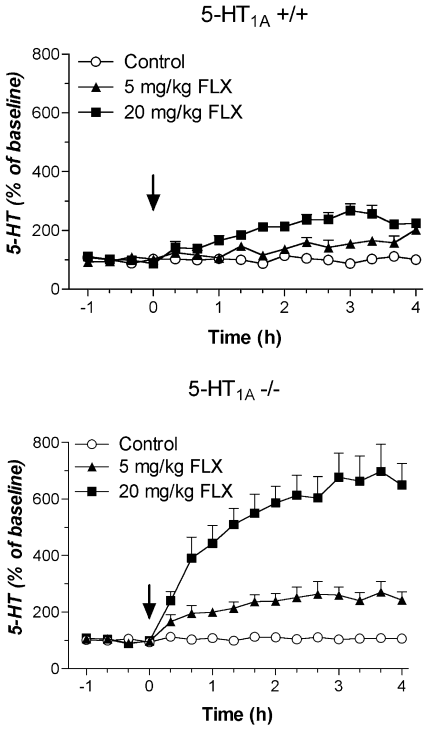

Fig. 5 Effects of the injection (arrows) of vehicle (control) or fluoxetine (FLX) on dialysate 5-HT in the DR of 5-HT$_{1A}$+/+ mice (top panel) and 5-HT$_{1A}$–/– mice (bottom panel). Vehicle injections had no effect in 5-HT$_{1A}$+/+ mice ($n = 6$) and 5-HT$_{1A}$–/– mice ($n = 7$). In contrast, fluoxetine at the doses of 5 mg/kg ($n = 4$ for 5-HT$_{1A}$+/+ mice and $n = 6$ for 5-HT$_{1A}$–/– mice) and 20 mg/kg ($n = 4$ for 5-HT$_{1A}$+/+ mice and $n = 7$ for 5-HT$_{1A}$–/– mice) increased dialysate 5-HT in a dose-dependent manner in both lines of mice. The effect of fluoxetine was more prominent in 5-HT$_{1A}$–/– mice, both at 5 mg/kg ($p < 0.02$) and 20 mg/kg ($p < 0.005$).

3 min, and its effects on dialysate 5-HT are displayed in Fig. 4. In the absence of citalopram in the perfusion fluid there was no effect of handling in either line of mice (Fig. 4, upper panel). However, when 1 μM citalopram was added (Fig. 4, lower panel), handling was without effect in 5-HT$_{1A}$+/+ mice, but dialysate 5-HT increased twofold in 5-HT$_{1A}$–/– mice, as shown by the significant effects of genotype ($p < 0.05$), time ($p < 0.00002$) and their interaction ($p < 0.01$).

Effects of fluoxetine

The effects of systemic fluoxetine on extracellular 5-HT in wild-type and 5-HT$_{1A}$ receptor knockout mice are depicted in Fig. 5. Two-way ANOVA showed that fluoxetine increased dialysate 5-HT in 5-HT$_{1A}$+/+ mice in a dose-dependent manner (Fig. 5, upper panel), as demonstrated by the significant effect of dose ($p < 0.0001$), time ($p < 0.0001$) and the interaction between both factors ($p < 0.001$). Fluoxetine also elevated dialysate 5-HT in 5-HT$_{1A}$–/– mice (Fig. 5, lower panel), as shown by the significant effect of dose ($p < 0.00005$), time ($p < 0.00001$) and the interaction between both factors ($p < 0.00001$). The effects of each dose of fluoxetine were greater in the 5-HT$_{1A}$ receptor knockout mice in comparison with their wild-type counterparts. Thus, the maximal effect of fluoxetine on dialysate 5-HT in the DR of 5-HT$_{1A}$–/– mice, in comparison with 5-HT$_{1A}$+/+ mice, was 1.6-fold greater after the dose of 5 mg/kg ($p < 0.02$), and 2.7-fold greater after the dose of 20 mg/kg ($p < 0.005$).

Discussion

The 5-HT$_{1A}$ receptor knockout mouse has been extensively characterized in terms of its anxiety-like behavior in several conflict tests (for review, see Toth 2003). However, little work has been devoted to studying the *in vivo* efflux of 5-HT, and so far this research has focused on forebrain regions. To our knowledge, this is the first report dealing with the *in vivo* efflux of 5-HT in the DR of 5-HT$_{1A}$ receptor knockout mice. The importance of the DR is further underscored by the fact that it is particularly responsive, in terms of changes in 5-HT efflux, to 5-HT$_{1A}$ receptor agonists (Casanovas *et al*. 1997) and stressful conditions (Adell *et al*. 1997). In the present work, the functional absence of 5-HT$_{1A}$ receptors in 5-HT$_{1A}$–/– mice was verified by the failure of 8-OH-DPAT to suppress the efflux of 5-HT. The disruption of the 5-HT$_{1A}$ receptor gene did not induce changes in the basal extracellular levels of 5-HT in the DR. This is coincident with previous findings obtained in forebrain regions, both *in vivo* (He *et al*. 2001; Knobelman *et al*. 2001b; Adell *et al*. 2003) and *in vitro* (Ramboz *et al*. 1998; Richer *et al*. 2002). The fact that 1 μM citalopram elevated dialysate 5-HT to a comparable extent in both lines of mice suggests that the efficiency of the 5-HT reuptake process, at least in the DR, is not altered by the lack of the 5-HT$_{1A}$ receptors. This is in line with previous work showing an absence of changes in the density of 5-HT transporter in the raphe nuclei of 5-HT$_{1A}$–/– mice (Ase *et al*. 2001; He *et al*. 2001). However, the present results do not seem to be in accordance with the increased 5-HT turnover seen in 5-HT$_{1A}$–/– mice (Ase *et al*. 2000). It is thus possible that the increased 5-HT turnover takes place in the cytoplasmic compartment and does not alter the release of the transmitter. The lack of changes in the basal efflux of 5-HT also agrees with an absence of major alterations in the firing rate of most DR serotonergic neurons (Richer *et al*. 2002; Adell *et al*. 2003), and provides further support to the view that 5-HT$_{1A}$ autoreceptors are not tonically activated under physiological conditions (Adell *et al*. 2002; Johnson *et al*. 2002). In mice, the absence of a tonic control of 5-HT$_{1A}$ receptors upon serotonergic neurons has been confirmed by Mannoury la Cour *et al*. (2001), who showed that the selective 5-HT$_{1A}$ receptor antagonist WAY 100635 did not affect the membrane potential of 5-HT cells in the DR. Alternatively, it could be expected that adaptive changes in the efficacy of 5-HT$_{1B}$ receptors might have developed in mice with a constitutive inactivation of the 5-HT$_{1A}$ receptor. For instance, using the selective 5-HT$_{1B}$ receptor agonists CP 93129 or CP 94253, a compensatory enhanced responsiveness of 5-HT$_{1B}$ autoreceptors in 5-HT$_{1A}$–/– mice has been observed (Ramboz *et al*. 1998; Knobelman *et al*. 2001a; Boutrel *et al*. 2002; but see Richer *et al*. 2002). However, these adaptive changes have not been found with the 5-HT$_{1B/1D}$ receptor agonist sumatriptan (Ramboz *et al*. 1998; Richer *et al*. 2002), which suggests that the function of 5-HT$_{1D}$ receptors is not changed in these mutant mice. In the present study, the efficacy of DR 5-HT$_{1B}$ receptors in reducing the local efflux of 5-HT is not altered in 5-HT$_{1A}$–/– mice, which agrees with the lack of changes in the density of this receptor in the DR (Ase *et al*. 2001) and argues against the development of compensatory changes of 5-HT$_{1B}$ receptors observed previously in mesencephalic slices (Ramboz *et al*. 1998). However, the occurrence of changes of the *in vivo* function of 5-HT$_{1D}$ receptors in the DR of 5-HT$_{1A}$ receptor knockout mice cannot be ruled out.

The existence of altered responses to different stressful conditions in 5-HT$_{1A}$–/– mice was also examined in the present study. In the absence of citalopram in the artificial CSF, both the injection of vehicle (control mice in fluoxetine experiments) and handling did not alter the basal efflux of 5-HT in either line of mice. In contrast, in the presence of 1 μM citalopram, the injection of vehicle, 8-OH-DPAT and handling markedly enhanced the 5-HT output in 5-HT$_{1A}$–/–, but not in 5-HT$_{1A}$+/+ mice. Recent research without the use of citalopram has also provided conflicting results. Thus, a saline injection did not alter striatal 5-HT output in both genotypes (He *et al*. 2001) whereas an open field exposure enhanced cortical but not hippocampal 5-HT efflux in mice lacking 5-HT$_{1A}$ receptors (Parsons *et al*. 2001). Therefore, the effects of stress on the efflux of 5-HT depend on how the

aversive stimulus is perceived, the brain region and the sampling conditions. In the DR the density of 5-HT uptake sites is very high (Hrdina *et al*. 1990; He *et al*. 2001) and presumably sufficient to dampen stress-related increases in spillover of released 5-HT into extracellular space. Therefore, it is likely that changes in the efflux of 5-HT would remain latent unless the reuptake process is partly inhibited. In the present study, the fact that 5-HT_{1A}–/– mice exhibit an enhanced response to stress indicates that 5-HT_{1A} receptors normally restrain the stress-induced enhancement of 5-HT efflux in the DR, but this becomes apparent only under conditions of decreased elimination of extracellular 5-HT. In a previous study we observed that a saline injection and a short handling elevated dialysate 5-HT in the raphe nuclei of the rat (Adell *et al*. 1997). It thus seems that, with 5-HT_{1A} receptors intact, the DR of the rat is more responsive to the effects of mild stressors than that of the mouse, although the basis for this species difference are not fully understood.

The control of the extracellular concentration of 5-HT in the DR of mice by 5-HT_{1A} receptors was further studied by investigating the effects of a challenge with the SSRI fluoxetine. In these experiments the use of citalopram was avoided to prevent the effects of fluoxetine from being masked. On the one hand, the systemic administration of fluoxetine to wild-type mice evoked increases in dialysate 5-HT lower than those observed in the DR of the rat (Malagié *et al*. 1995; Rutter *et al*. 1995; Hervás and Artigas 1998), which may be accounted for by species differences in the density of 5-HT_{1A} receptors. On the other hand, the absence of 5-HT_{1A} receptors resulted in an enhanced fluoxetine-induced response of DR 5-HT in 5-HT_{1A}–/– mice, consistent with the lack of fluoxetine-induced inhibition of 5-HT cell firing in these mice (Adell *et al*. 2003). Using mutant mice of different genetic backgrounds, comparable results have also been obtained in forebrain regions (He *et al*. 2001; Knobelman *et al*. 2001a,b; Parsons *et al*. 2001). The enhanced response to systemic fluoxetine administration in the DR of 5-HT_{1A}–/– mice appears contradictory with the comparable increase in both lines of mice after local citalopram administration (see above). This discrepancy can be explained simply by the evidence that, at 1 μM, citalopram only partially blocks the avid reuptake process and this effect is limited to an area right next to the probe. Thus, discharge and release of 5-HT may be virtually unaltered. Additionally, 5-HT_{1A} receptors in the forebrain also control the efflux of 5-HT in the DR (Celada *et al*. 2001) and the local perfusion of a SSRI into the DR would not influence those receptors. In contrast, the increased responsiveness of 5-HT_{1A}–/– mice to systemic fluoxetine most likely reflects the absence of the inhibitory mechanism(s) dependent upon 5-HT_{1A} autoreceptors. As a matter of fact, the pharmacological blockade of this inhibitory process mediated by 5-HT_{1A} autoreceptors augments the SSRI-induced increase in 5-HT neurotransmission (Artigas *et al*. 1996; Romero and Artigas 1997). Thus, the potentiation of the effects of fluoxetine in mice constitutively deprived of 5-HT_{1A} receptors provides further support for the hypothesis that the absence of 5-HT_{1A} receptor-induced negative feedback increases the efficiency of SSRIs (Artigas *et al*. 1994; Blier and Bergeron 1995; Perez *et al*. 1997). Further research is needed to ascertain the contribution of postsynaptic 5-HT_{1A} receptors to the mechanism of action of systemic SSRIs.

In conclusion, 5-HT efflux in the DR of 5-HT_{1A} receptor knockout mice was enhanced in response to stress and fluoxetine without apparent modification of the function of 5-HT_{1B} receptors.

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