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Serotonin transporter in substance P (neurokinin 1) receptor knock-out mice

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Abstract

We recently demonstrated that mice lacking the gene for substance P (neurokinin 1) receptors ($NK_1-/-$) show improved cortical dialysate serotonin (5-HT) responses to paroxetine [J. Neurosci. 21 (2001) 8188]. To test for changes that may involve the 5-HT transporter (5-HTT) in these mutant mice, in vivo/in vitro studies were performed. Autoradiographic quantification of 5-HTT was performed: [^{3}H]citalopram binding did not reveal any modification of 5-HT binding sites in the dorsal raphe nucleus (DRN) of wild-type $NK_1+/+$ control and mutant $NK_1-/-$ mice. These results were further confirmed by 5-HTT mRNA quantification using competitive reverse transcription and polymerase chain reaction (RT-PCR) assay, which showed similar messenger levels in the DRN of both mice genotypes. The functional status of 5-HTT in vivo was tested by using the zero net flux method of quantitative microdialysis in two serotonergic nerve terminal regions, the frontal cortex and ventral hippocampus, of wild-type $NK_1+/+$ and $NK_1-/-$ mice. Neither basal extracellular 5-HT levels nor the 5-HT extraction fraction of the probe (E_d an index of 5-HT uptake in vivo) differed between wild-type and mutant mice in the two brain regions studied. These results suggest that no compensatory response to the constitutive deletion of the tachykinin NK_1 receptor involving changes in the activity of the selective 5-HT transporter occurred in the DRN, frontal cortex and ventral hippocampus in mice.

Keywords: Substance P (neurokinin 1) receptor; Serotonin (5-HT) transporter; In vivo intracerebral microdialysis; Knock-out, mice

1. Introduction

Several antagonists of the substance P (neurokinin 1) receptor (NK_1) are in an advanced stage of clinical development as a novel approach for treating major depressive disorder (Kramer et al., 1998, 2004). These molecules are active in various preclinical assays of antidepressant activity (Rupniak, 2002). They are claimed to display comparable clinical efficacy to that measured with the selective serotonin reuptake inhibitors (SSRIs). We recently explored the functional status of the brain serotonergic system by using in vivo intracerebral microdialysis in mice lacking tachykinin NK_1 receptors ($NK_1-/-$) and found that a single systemic administration of the SSRI paroxetine induced a larger increase in extracellular levels of serotonin (5-hydroxytryptamine, 5-HT) in the frontal cortex of freely moving $NK_1-/-$ than in wild-type ($NK_1+/+$) mice (Froger et al., 2001). This result suggests that, in wild-type mice, the activation of tachykinin NK_1 receptors by endogenous substance P limits the effects of SSRIs on cortical dialysate 5-HT levels in mice. Accordingly, we also found that the constitutive lack of tachykinin NK_1 receptors is associated with a down-regulation/functional desensitization of 5-HT_{1A}

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autoreceptors, resembling that induced by chronic treatment with SSRI antidepressant drugs (Blier and De Montigny, 1983; Jolas et al., 1994; Le Poul et al., 1995). In our study, double immunocytochemical labeling experiments failed to demonstrate the presence of tachykinin NK_1 receptors on cell bodies and dendrites of serotonergic neurons located in the dorsal raphe nucleus (DRN) of wild-type mice. It is thus unlikely that direct interactions between the serotonergic and substance P-ergic systems occur in this brain region.

To strengthen the interpretation of the above-described microdialysis data, it is important to verify whether or not compensatory alterations involving the selective serotonin transporter (5-HTT) took place in the brain during the development of these $NK_1-/-$ mice generated by homologous recombination (De Felipe et al., 1998). Indeed, several cross-regulations between brain neuropeptide and neurotransmitter systems have already been observed in genetically modified animals. For example, in mice lacking the μ-opioid receptor gene, a small reduction in [^{3}H]nitrobenzylthioinosine binding to adenosine transporters was detected in brains (Bailey et al., 2002). In addition, by using the zero net flux method of quantitative microdialysis, it has been shown that basal dopamine dynamics or cocaine-evoked dopamine levels are altered in projection areas of mesoaccumbens dopamine neurons in 5-HT_{1B} receptor knock-out mice (Shippenberg et al., 2000). These data suggest that deletion of the 5-HT_{1B} receptor gene lead to alterations of dopamine uptake in the nucleus accumbens. It is hypothesized that these alterations in presynaptic neuronal activity are compensatory responses to constitutive deletion of 5-HT_{1B} receptors.

In the present study, the characteristics of 5-HTT were assessed in $NK_1-/-$ mice compared to their wild-type NK_1+/+ controls using various methods: (i) autoradiographic mapping of specific 5-HTT labeling by [^{3}H]citalopram within the DRN; (ii) quantification by competitive reverse transcription and polymerase chain reaction (RT-PCR) of specific 5-HTT mRNA in the DRN area; and finally (iii) in vivo quantitative microdialysis in the frontal cortex and the ventral hippocampus of mice. These investigations are important for knowing whether these $NK_1-/-$ mutant mice can be viewed as a model of life-long treatment with tachykinin NK_1 receptor antagonists, which are putative antidepressant drugs (Rupniak, 2002).

2. Materials and methods

2.1. Animals

The founders of the wild-type and mutant colonies used in the present study were the product of heterozygous mating made at the animal facility of University College, London (De Felipe et al., 1998). These founders were shipped to France and their offspring were bred and reared as already described (Froger et al., 2001). Wild-type and $NK_1-/-$ mice were the product of mating between heterozygous NK_1+/− couples raised on a C57BL/6 genetic background. Group-housed mice were kept in standard cages under a 12-h light/12-h dark cycle with light onset at 7 h. Mice had free access to food and water. All procedures used in these studies were performed under the guidelines of the French Ministry of Agriculture for experiments with laboratory animals (law no. 87848) and were approved by the appropriate local committee.

2.2. In vitro quantification of 5-HT transporter

Mouse were killed by decapitation and the brains from wild-type and mutant mice were rapidly removed, immediately frozen in isopentane chilled at −30 °C by dry ice, then stored at −20 °C. For the quantification of the specific 5-HTT mRNA by competitive RT-PCR, after the removal of the brain, DRN was rapidly dissected before freezing in isopentane chilled at −30 °C by dry ice, then stored at −80 °C.

2.2.1. Autoradiographic quantification of specific 5-HTT labeling

Coronal sections (20 μm) were cut at −20 °C, thaw-mounted onto gelatin-coated slides and then stored at −20 °C until use. Autoradiographic quantification of 5-HTT was measured in the DRN of NK_1+/+ control ($n=6$) and $NK_1-/-$ mutant ($n=6$) mice, using the selective radioligand [^{3}H]citalopram. Brain sections were first brought to room temperature and then preincubated at 25 °C for 15 min in 50 mM Tris–HCl, pH 7.4, supplemented with 120 mM NaCl–5 mM KCl. Sections were incubated with 0.7 nM [^{3}H]citalopram (85 Ci/mMol) in the same buffer for 2 h at 25 °C. Non-specific binding was estimated in adjacent sections incubated with 0.7 nM [^{3}H]citalopram and 10 μM fluoxetine in the same buffer. Sections was then washed four times for 5 min each in the same buffer at 4 °C, and briefly immersed in ice-cold distilled water. The slides were dried in a stream of cold air and exposed to a [^{3}H] Fuji imaging plate BAS-TR2040 (Fujifilm). After 2 weeks of exposure, the imaging plate was scanned using a phosphoimager FLA2000 (Fuji). The scanned image was converted into a computerized image using Aïda 2.1 software and the optical density was measured and converted to fmol [^{3}H]citalopram specifically bound per mg of tissue according to a [^{3}H] standard scale (Amersham Pharmacia Biotech) for 15–20 sections per mouse.

2.2.2. Quantification of specific 5-HTT mRNA by competitive RT-PCR

The method used to measure mRNA was based on a competitive reverse transcriptase-polymerase chain reaction (RT-PCR) technique (Siebert and Larrick, 1992), in which mRNAs of analyzed gene are reverse-transcribed and amplified in the presence of a homologous deleted internal standard mRNA.

Quantitative determination of 5-HTT receptor mRNA in the raphe area was performed as described by Le Poul et al. (2000), using a RT-PCR Access System Kit (Promega, Madison, WI, USA). Reverse transcription (45 min at 48 °C) proceeded with 0.2 μg of total tissue RNA in the presence of standard deleted RNA at increasing dilutions (5×10^{-6} to 10^{-7}). The sequences of the upstream and downstream oligonucleotide primers were 5′ -GGATCC-CTGCTCACACTG-3′ (nucleotides 1541-1548), and 5′ -AATGTGTCGTAAGTACGC-3′ (nucleotides 2008-1990), respectively. PCR amplification was performed with 1–2 units of Tfl DNA polymerase, 1 m M $MgSO_4$ and 1 pg/μl of each primer for 30 cycles (1 min at 95 °C, 1 min at 58 °C and 1 min at 72 °C). After electrophoretic separation in 2% agarose gel stained with 4% ethidium bromide, both standard and tissue RT-PCR products were quantified with gel analyzer software (NIH 1.6).

2.3. In vivo quantification: zero net flux method of quantitative microdialysis

Concentric dialysis probes were made of cuprophan fibers and constructed as described previously (Malagié et al., 2001). All probes (× 0.30 mm OD) had an active length of 1.6 mm. Male wild-type NK_1+/+ and mutant NK_1 −/− mice (10–12 weeks old, 25–30 g body weight) were anesthetized with chloral hydrate (400 mg/kg, i.p.). The microdialysis probe was implanted either in the frontal cortex or ventral hippocampus according to the mouse brain atlas of Franklin and Paxinos (1997) (coordinates from bregma (in mm), frontal cortex, $A = +2.0$, $L = +1.2$, $V = -1.6$; ventral hippocampus, $A = -2.8$, $L = -3.0$, $V = -4.0$). The next day, after a ~ 20 h recovery from the surgery, the probe was continuously perfused with microdialysis medium (composition in mM: NaCl 147, KCl 3.5, $CaCl_2$ 1.26, $MgCl_2$ 1.2, NaH_2PO_4 1.0, $NaHCO_3$ 25.0, pH 7.4) at a flow rate of 1.3 μl/min, using a CMA/100 pump (Carnegie Medicine, Stockholm, Sweden). Dialysate samples were collected every 15 min in small Eppendorf tubes for the measurement of their 5-HT contents using high-performance liquid chromatography (XL-ODS, 4.6 × 7.0 mm, particle size 3 μm; Beckman) coupled to an amperometric detector (1049A, Hewlett-Packard, Les Ulis, France) as previously described (Malagié et al., 2001). Usually, four fractions were collected to determine basal values (means ± S.E.M.) before local perfusion of a single dose of serotonin. The limit of sensitivity for 5-HT was ~ 0.5 fmol/sample (signal-to-noise ratio = 2).

The zero net flux method of quantitative microdialysis was performed in the frontal cortex and ventral hippocampus of wild-type NK_1+/+ and mutant NK_1 −/− mice. The dialysate 5-HT concentrations (C_{out}) obtained during perfusion of the various concentrations of 5-HT (C_{in}: 0, 5, 10 and 20 nM) were used to construct a linear equation for each animal. The net change in 5-HT ($C_{in} - C_{out}$) was regressed against C_{in}. Extracellular 5-HT levels ([5-HT]ext) and the extraction fraction of the probe (Ed) were determined as described by Parson and Justice (1994). [5-HT]ext is equal to the C_{in} concentration at which $C_{in} - C_{out} = 0$ and corresponds to equilibrium conditions. The extraction fraction (Ed) is the slope of the linear regression and has been shown to provide an estimate of changes in transporter-mediated 5-HT uptake (Justice, 1993; Parson and Justice, 1994; Shippenberg et al., 2000; Gardier et al., 2003). At the end of the experiments, placement of microdialysis probes was verified histologically.

2.4. Statistical analysis

Statistical analyses were performed using the computer software StatView 4.02 (Abacus Concepts, Berkely, CA, USA). For the zero net flux experiment, a one-way analysis of variance (ANOVA) was used to assess the effects of genotype on [5-HT]ext and E_d in each brain region of wild-type and NK_1 −/− mutant mice. The significance level was set at $P < 0.05$.

3. Results

3.1. In vitro quantification

3.1.1. 5-HTT binding sites

5-HTT labeling by the selective radioligand [^{3}H]citalopram was measured in the DRN of brain sections from the two groups of mice (Fig. 1). Specific 5-HTT labeling was 365.8 ± 0.68 fmol/mg tissue (mean ± S.E.M., $n = 6$) in brain sections from NK_1+/+ mice compared to 335.6 ± 1.8 fmol/

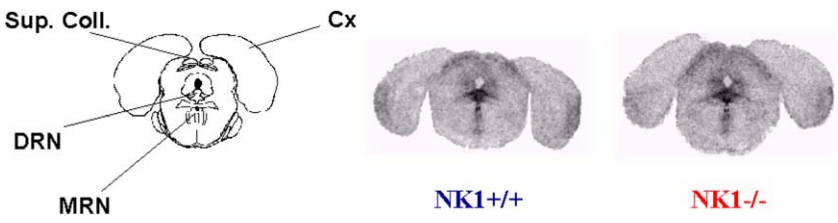

Fig. 1. Comparison of serotonin transporter (5-HTT) labeling by the selective serotonin reuptake inhibitor [^{3}H]citalopram in the dorsal raphe nucleus (DRN) of brain sections from wild-type (NK_1+/+) vs. tachykinin NK_1 receptor knock-out (NK_1 −/−) mice. MRN: median raphe nucleus.

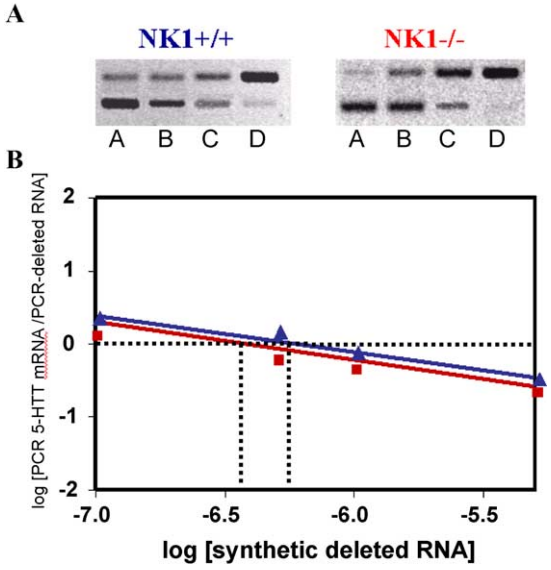

Fig. 2. Quantification of serotonin-transporter mRNA by competitive RT-PCR in the DRN of wild-type (NK_1+/+) vs tachykinin NK_1 receptor knock-out ($NK_1 -/-$) mice. (A) Electrophoretic separation in a 2% agarose gel stained with ethidium bromide of PCR products from $NK_1 -/-$ mutant mice vs wild-type NK_1+/+ control mice. (B) Plot for the quantification of PCR products: the logarithmic ratio of the amounts (OD measurements) of the specific mRNA (484 bp) over those of the synthetic deleted RNA (400 bp) is plotted as a function of the logarithm of each serial dilution of the synthetic deleted RNA. (Δ) represents the log [PCR 5-HTT mRNA of NK_1+/+/PCR-deleted RNA], whereas (♦) represents the log [PCR 5-HTT mRNA of $NK_1 -/-$/PCR-deleted RNA]. The intersection of each line with the *x*-axis gives the equivalent dilution of the synthetic deleted RNA, thus the equivalent amount of specific 5-HTT mRNA in the mouse dorsal raphe nucleus.

mg tissue (mean ± S.E.M., $n=6$,) in brain sections from mutant $NK_1 -/-$ mice. The difference in 5-HTT labeling between $NK_1 -/-$ and NK_1+/+ mice was not statistically significant.

3.1.2. 5-HTT mRNA

The electrophoretic separation in a 2% agarose gel stained with ethidium bromide of PCR products did not reveal any differences between NK_1+/+ control and $NK_1 -/-$ mutant mice (Fig. 2A). The logarithmic ratio of the amounts (OD measurements) of the specific mRNA over those of the synthetic deleted RNA, plotted as a function of the logarithm of each serial dilution of the synthetic deleted RNA, was identical in both mice genotypes (Fig. 2B). Quantitative determination by competitive RT-PCR showed that the 5-HTT mRNA levels in the raphe area were not significantly different in $NK_1 -/-$ mutant mice (0.51 ± 0.12 attomoles specific mRNA/μg of total mRNA, mean ± S.E.M., $n=5$) compared to NK_1+/+ control mice (0.44 ± 0.21 attomoles specific mRNA/μg of total mRNA mean ± S.E.M., $n=5$).

3.2. In vivo quantification

Fig. 3 shows the experimental protocol of the zero net flux method of quantitative microdialysis performed in wild-type NK_1+/+ control and mutant $NK_1 -/-$ mice. The zero net flux plot of the mean ± S.E.M. change in perfusate 5-HT concentration (C_{in}-C_{out}) is expressed as a function of perfusate 5-HT concentration (C_{in}) in the frontal cortex and ventral hippocampus of wild-type NK_1+/+ and mutant $NK_1 -/-$ mice (Figs. 4 and 5). The *y*-intercept corresponds to dialysate 5-HT levels that would be obtained in a conventional dialysis experiment. A summary of the results obtained for extracellular 5-HT levels and the slope of the linear regression (Ed) is given in Table 1.

In the frontal cortex, no differences were observed between the two genotypes regarding Ed (wild-type: 0.44 ± 0.10; $NK_1 -/-$: 0.53 ± 0.15; $F(1,8]=0.376$; $P>0.55$: Fig. 4A) or for dialysate 5-HT levels (*y*-intercept

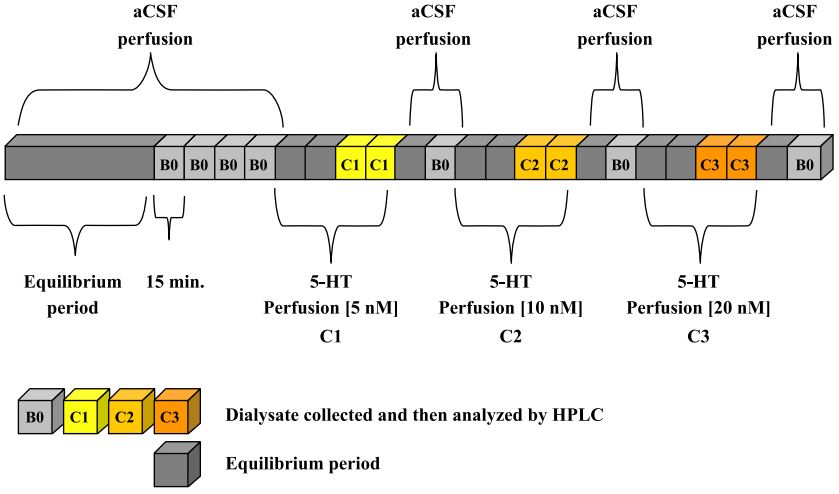

Fig. 3. Experimental protocol of the zero net flux method of quantitative microdialysis performed with wild-type (NK_1+/+) vs tachykinin NK_1 receptor knock-out ($NK_1 -/-$) mice. Each cube represents a 15-min period of dialysate collection. B0: basal extracellular levels of 5-HT; C1: the lowest 5-HT concentration perfused, i.e., 5 nM perfused in 30 min.; C2: the intermediate 5-HT concentration perfused, i.e., 10 nM perfused in 30 min.; C3: the highest 5-HT concentration perfused, i.e., 20 nM perfused in 30 min.

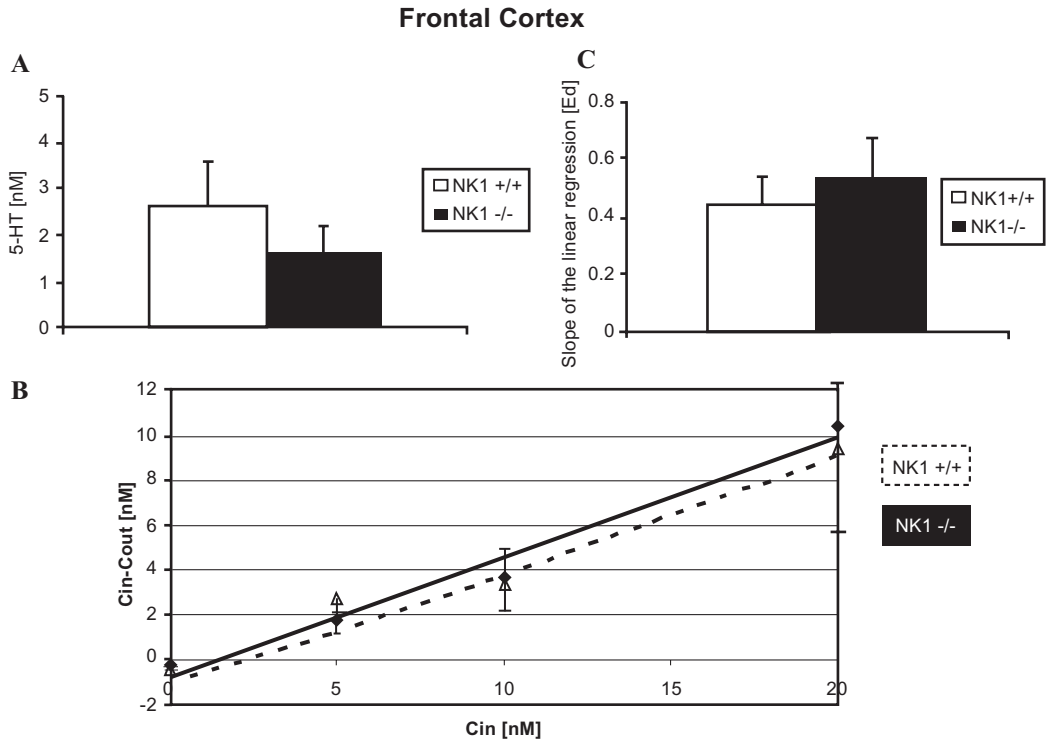

Fig. 4. Zero net flux plot of basal 5-HT dynamics in the frontal cortex of wild-type NK_1+/+ control and mutant $NK_1 -/-$ mice. Plots show the mean ± S.E.M. gain or loss of 5-HT ($C_{in} - C_{out}$) as a function of C_{in} (0, 5, 10, 20 nM of 5-HT) and the average linear regression of the data for the two genotypes. The C_{in} at which $C_{in} - C_{out} = 0$ equals the basal extracellular 5-HT concentration ([5-HT]ext), and the slope of linear regression corresponds to the extracellular fraction of the probe (E_d). The *y*-intercept corresponds to dialysate levels that would be obtained in a conventional dialysis experiment. (A) means ± S.E.M. of basal [5-HT]ext for $n = 6$ mice per group; (B) *y*-intercept and (C) means ± S.E.M. of E_d for $n = 6$ mice per group. These parameters were not significantly different in the two genotypes studied.

in nM: wild-type: 1.0 ± 0.03; $NK_1 -/-$: 0.76 ± 0.11; $P > 0.69$: Fig. 4B). Similarly, no differences were observed between genotypes regarding basal extracellular levels of 5-HT (mean ± S.E.M., $n = 6$, in nM: wild-type: 2.60 ± 1.0; $NK_1 -/-$: 1.6 ± 0.6; $F(1,8) = 0.414$; $P > 0.538$: Fig. 4C).

In the ventral hippocampus, no differences were observed between the two genotypes regarding Ed (wild-type: 0.58 ± 0.07; $NK_1 -/-$: 0.43 ± 0.12; $F(1,9] = 0.389$; $P > 0.38$: Fig. 5A) or for dialysate 5-HT levels (*y*-intercept in nM: wild-type: 0.60 ± 0.02; $NK_1 -/-$: 0.31 ± 0.11; $P > 0.73$: Fig. 5B). Similarly, no differences were observed between genotypes regarding basal extracellular levels of 5-HT (mean ± S.E.M., $n = 6$, in nM: wild-type: 1.04 ± 0.39; $NK_1 -/-$: 1.29 ± 0.46; $F(1,9) = 0.180$; $P > 0.68$: Fig. C).

Table 1
Basal 5-HT dynamics in wild-type and tachykinin $NK_1 -/-$ receptor knockout mice

Genotype	Frontal cortex	Ventral hippocampus
Wild-type mice	Basal [5-HT]ext (nM): 2.60 ± 1.0 E_d: 0.44 ± 0.10 Dialysate (*y*-intercept) (nM) 1.0 ± 0.03	Basal [5-HT]ext (nM): 1.04 ± 0.39 E_d: 0.58 ± 0.07 Dialysate (*y*-intercept) (nM): 0.60 ± 0.02
NK_1 knockout −/− mice	Basal [5-HT]ext (nM): 1.62 ± 0.6 E_d: 0.53 ± 0.15 Dialysate (*y*-intercept) (nM): 0.76 ± 0.11	Basal [5-HT]ext (nM): 1.29 ± 0.46 E_d: 0.43 ± 0.12 Dialysate (*y*-intercept) (nM): 0.31 ± 0.11

These were no statistically significant differences between genotypes. Values for [5-HT]ext, E_d and dialysate 5-HT levels were obtained for each animal by constructing a linear equation of dialysate 5-HT levels obtained during local perfusion of various concentrations of 5-HT as described in Materials and methods. Values represent the means ± S.E.M. of $n = 6$ determinations per group.

4. Discussion

5-HT reuptake via 5-HTT is the major mechanism responsible for 5-HT inactivation in the central nervous system. In brain tissues, 5-HTT is essentially localized on serotonergic neurons, at the level of the somas, dendrites, axons and nerve terminals. In the mouse brain, high levels of 5-HTT mRNA were detected in all brain stem raphe nuclei where serotonergic cell bodies are located (Bengel et al., 1997). However, differences in the regulation of the selective 5-HT transporter (5-HTT) in serotonergic nerve terminal brain regions (cortex, hippocampus) versus raphe nuclei containing the cell bodies of 5-HT neurons have not been clearly demonstrated because, to our knowledge, 5-HTT mRNA is undetectable in forebrain regions. Thus, autoradiographic measurements of serotonin transporter obtained by using the radioligand [^{3}H]citalopram in mice showed that 5-HTT binding protein density in forebrain

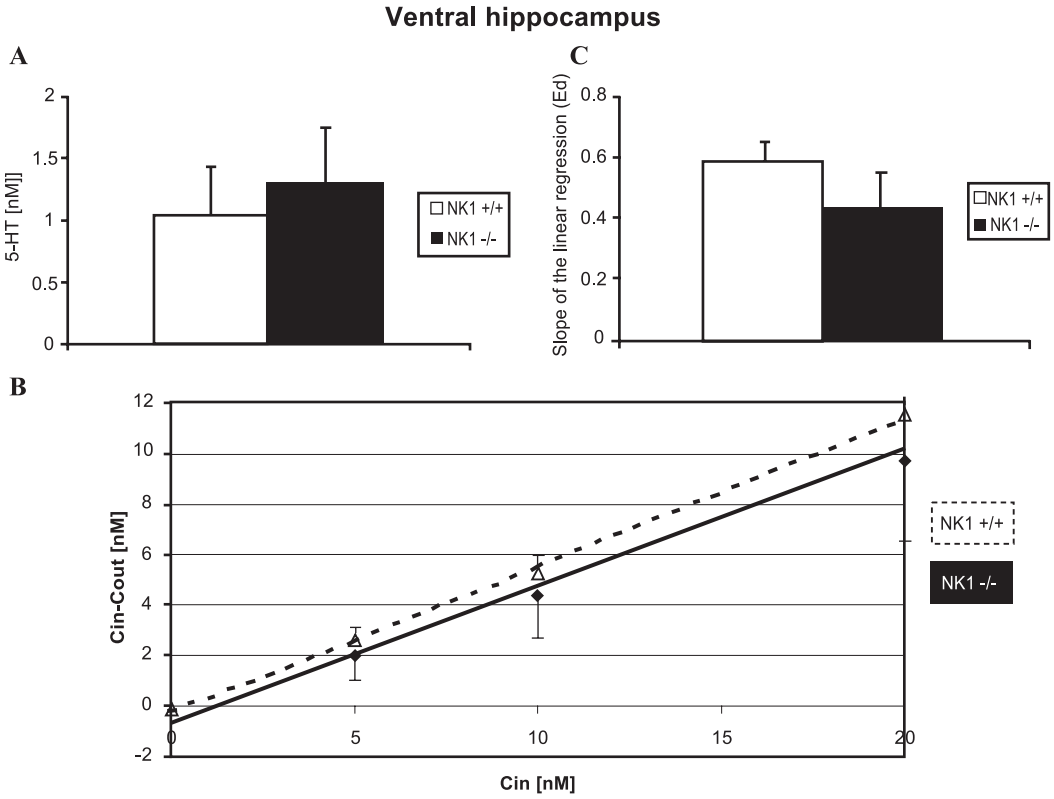

Fig. 5. Zero net flux plot of basal 5-HT dynamics in the ventral hippocampus of wild-type NK_1+/+ control and mutant NK_1 −/− mice. The plots show the mean ± S.E.M. gain or loss of 5-HT ($C_{in} - C_{out}$) as a function of C_{in} (0, 5, 10, 20 nM of 5-HT) and the average linear regression of the data in the two genotypes. The C_{in} at which $C_{in} - C_{out} = 0$ equals the basal extracellular 5-HT concentration (([5-HT]ext), and the slope of linear regression corresponds to the extracellular fraction of the probe (E_d). The *y*-intercept corresponds to dialysate 5-HT levels that would be obtained in a conventional dialysis experiment. No differences were observed between the two genotypes studied regarding (A) means ± S.E.M. of basal ([5-HT]ext for $n = 6$ mice per group; (B) *y*-intercept and (C) means ± S.E.M. of E_d for $n = 6$ mice per group.

regions was very low, i.e., approximately one-fourth of that measured in raphe nuclei (Ase et al., 2001). In the present study, we did not find differences in the 5-HTT binding density between forebrain regions of the two genotypes.

The present in vivo/in vitro studies demonstrate that constitutive deletion of the tachykinin NK_1 receptor is not associated with any alteration in the serotonin transporter responsible for 5-HT reuptake: 5-HT transporter binding sites specifically labeled with [^{3}H]citalopram were identical in the DRN of both wild-type and mutant mice; 5-HTT mRNA levels in the DRN were unchanged by the mutation; and basal extracellular 5-HT levels, dialysate 5-HText or the 5-HT extraction fraction of the probe (E_d, an in vivo index of 5-HT uptake) in the frontal cortex and ventral hippocampus did not differ between wild-type and mutant mice.

We previously reported that the lack of tachykinin NK_1 receptors in mice was associated with a functional desensitization of somatodendritic 5-HT_{1A} autoreceptors (Froger et al., 2001). Numerous studies performed in rats have shown that chronic antidepressant drug administration leads to a desensitization of these 5-HT_{1A} autoreceptors (Chaput et al., 1991; Le Poul et al., 1997, 2000; see Hjorth et al., 2000 for a review), with modification neither in 5-HTT mRNA levels nor in 5-HTT binding sites density (Spurlock et al., 1994; Benmansour et al., 1999; Le Poul et al., 2000), as found in this present study. Moreover, two different experimental conditions (SSRIs treatment or tachykinin NK_1 receptor gene deletion) that led to 5-HT_{1A} autoreceptor desensitization did not modify the expression and the function of the brain 5-HTT.

Serotonergic raphe neurons do not seem to be under a tonic inhibitory influence of endogenous 5-HT acting through its binding to somatodendritic 5-HT_{1A} receptors. Negative results have been obtained by using two different approaches. First, by using a pharmacological approach, it has been shown in behaving cats that 5-HT_{1A} receptor antagonists increase the firing of 5-HT neurons in raphe nuclei, but only in periods when 5-HT neurons are most active (Fornal et al., 1996). Consistent with these findings, intracerebral in vivo microdialysis studies reported no detectable increase in 5-HT neurotransmission in response to 5-HT_{1A} receptor antagonists under normal conditions in rodents (see Toth, 2003 for a review). Second, by using a genetic approach, Ramboz et al. (1998) found no significant difference between mice lacking 5-HT_{1A} receptors and their wild-type controls either in the electrically evoked release of 5-[^{3}H]HT from mesencephalic slices or in the total tissue content of 5-HT in numerous brain regions. Thus, 5-HT

release does not appear to be tonically regulated by somatodendritic 5-HT_{1A} receptors under basal physiological conditions.

Our previous microdialysis study with $NK_1 -/-$ mice demonstrated that a single systemic administration of paroxetine induced a larger increase in the extracellular 5-HT levels in the frontal cortex of mutants compared to wild-type NK_1+/+ mice (Froger et al., 2001). In these experiments, the paroxetine-induced increase in dialysate 5-HT levels may reflect either an increase in 5-HT release or a decrease in 5-HT reuptake, or both. This effect was shown to be reinforced in mutant mice. The zero net flux method of quantitative microdialysis provides an indirect in vivo index of changes in transporter-mediated neurotransmitter uptake in rats (Thompson et al., 2000). It has already proven to be a useful technique to compare the neurotransmitter reuptake capacity of wild-type and mutant mice, such as 5-HT_{1B} receptor knock-out mice (Shippenberg et al., 2000 for dopamine; our group for 5-HT, Gardier et al., 2003) or dopamine transporter knock-out mice (Jones et al., 1999). The present results suggest that the potentiation of the paroxetine-induced increase in extracellular 5-HT levels we previously described in the frontal cortex of mutant $NK_1 -/-$ mice (Froger et al., 2001) is unlikely to be related to changes in 5-HT transporter-mediated clearance of extracellular 5-HT. This potentiated response could be related to somatodendritic 5-HT_{1A} receptor downregulation (see above).

No developmental compensatory response involving 5-HTT seems to occur following the constitutive deletion of the tachykinin NK_1 receptor gene in this mouse's strain. Conversely, the present microdialysis data demonstrate that tachykinin NK_1 receptor deletion affects presynaptic 5-HT neuronal function similarly in the frontal cortex and ventral hippocampus, two different serotonergic nerve terminal brain regions. Indeed, the frontal cortex and the ventral hippocampus are preferentially innervated by the dorsal and the median raphe nuclei, respectively (Jacobs and Azmitia, 1992; Hervas et al., 1998, 2000). The lack of effect on basal extracellular 5-HT levels in the two brain regions studied suggests the absence of a tonically active tachykinin NK_1 receptor control of the activity of DRN-cortical and MRN-hippocampal serotonergic neurons in mice.

Taken together, our data indicate that constitutive deletion of tachykinin NK_1 receptors does not alter serotonin uptake activity and serotonin clearance from the extracellular compartment in two brain regions, suggesting that direct functional interactions between tachykinin NK_1 receptors and serotonin transporters are unlikely to occur in the mouse brain. This result may have interesting therapeutic implications for the treatment of major depressive disorders, since a better efficacy in the treatment of this disease may be achieved by combining a tachykinin NK_1 receptor antagonist and a selective serotonin reuptake inhibitor.

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References

Ase, A.R., Reader, T.A., Hen, R., Riad, M., Descarries, L., 2001. Regional changes in density of serotonin transporter in the brain of 5-HT_{1A} and 5-HT_{1B} knockout mice, and of serotonin innervation in the 5-HT_{1B} knockout. J. Neurochem. 78, 619–630.

Bailey, A., Matthes, H., Kieffer, B., Slowe, S., Hourani, S.M., Kitchen, I., 2002. Quantitative autoradiography of adenosine receptors and NBTI-sensitive adenosine transporters in the brains and spinal cords of mice deficient in the μ-opioid receptor gene. Brain Res. 943, 68–79.

Bengel, D., Johren, O., Andrews, A.M., Heils, A., Mossner, R., Sanvitto, G.L., Saavedra, J.M., Lesch, K.P., Murphy, D.L., 1997. Cellular localization and expression of the serotonin transporter in mouse brain. Brain Res. 778, 338–345.

Benmansour, S., Cecchi, M., Morilak, D.A., Gerhardt, G.A., Javors, M.A., Gould, G.G., Frazer, A., 1999. Effects of chronic antidepressant treatments on serotonin transporter function, density, and mRNA level. J. Neurosci. 19, 10494–10501.

Blier, P., De Montigny, C., 1983. Electrophysiological investigations on the effect of repeated zimelidine administration on serotoninergic neurotransmission in the rat. J. Neurosci. 3, 1270–1278.

Chaput, Y., de Montigny, C., Blier, P., 1991. Presynaptic and postsynaptic modifications of the serotonin system by long-term administration of antidepressant treatments. An in vivo electrophysiologic study in the rat. Neuropsychopharmacology 5, 219–229.

De Felipe, C., Herrero, J.F., O'Brien, J.A., Palmer, J.A., Doyle, C.A., Smith, A.J.H., Laird, J.M.A., Belmonte, C., Cervero, F., Hunt, S.P., 1998. Altered nociception, analgesia and aggression in mice lacking the receptor for substance P. Nature 392, 394–397.

Fornal, C.A., Metzler, C.W., Gallegos, R.A., Veasey, S.C., McCreary, A.C., Jacobs, B.L., 1996. WAY-100635, a potent and selective 5-hydroxytryptamine1A antagonist, increases serotonergic neuronal activity in behaving cats: comparison with (S)-WAY-100135. J. Pharmacol. Exp. Ther. 278, 752–762.

Franklin, K.B., Paxinos, G., 1997. The Mouse Brain in Stereotaxic Coordinates. Academic Press, Toronto.

Froger, N., Gardier, A.M., Moratalla, R., Alberti, I., Lena, I., De Felipe, C., Rupniak, N.M.J., Hunt, S.P., Jacquot, C., Hamon, M., Lanfumey, L., 2001. 5-HT_{1A} autoreceptor adaptive changes in substance P (NK_1) receptor knock-out mice mimic antidepressant-induced desensitisation. J. Neurosci. 21, 8188–8197.

Gardier, A.M., David, D.J., Jego, G., Przybylski, C., Jacquot, C., Durier, S., Gruwez, B., Douvier, E., Beauverie, P., Poisson, N., Hen, R., Bourin, M., 2003. Effects of chronic paroxetine treatment on dialysate serotonin in 5-HT_{1B} receptor knockout mice. J. Neurochem. 86, 13–24.

Hervas, I., Bel, N., Fernandez, A.G., Palacios, J.M., Artigas, F., 1998. In vivo control of 5-hydroxytryptamine release by terminal autoreceptors in rat brain areas differentially innervated by the dorsal and median raphe nuclei. Naunyn-Schmiedeberg's Arch. Pharmacol. 358, 315–322.

Hervas, I., Queiroz, C.M., Adell, A., Artigas, F., 2000. Role of uptake inhibition and autoreceptor activation in the control of 5-HT release in the frontal cortex and dorsal hippocampus of the rat. Br. J. Pharmacol. 130, 160–166.

Hjorth, S., Bengtsson, H.J., Kullberg, A., Carlzon, D., Peilot, H., Auerbach, S.B., 2000. Serotonin autoreceptor function and antidepressant drug action. J. Psychopharmacol. 14, 177–185.

Jacobs, B.L., Azmitia, E.C., 1992. Structure and function of the brain serotonin system. Physiol. Rev. 72, 165–229.

Jolas, T., Haj-Dahmane, S., Kidd, E.J., Langlois, X., Lanfumey, L., Fattaccini, C.M., Vantalon, V., Laporte, A.M., Adrien, J., Gozlan, H., Hamon, M., 1994. Central pre- and postsynaptic 5-HT_{1A} receptors in rats treated chronically with a novel antidepressant, ericlamine. J. Pharmacol. Exp. Ther. 268, 1432–1443.

Jones, S.R., Gainetdinov, R.R., Caron, M.G., 1999. Application of microdialysis and voltammetry to assess dopamine functions in genetically altered mice: correlation with locomotor activity. Psychopharmacology (Berl.) 147, 30–32.

Justice, J.B., 1993. Quantitative microdialysis of neurotransmitters. J. Neurosci. Methods 48, 261–276.

Kramer, M.S., Cutler, N., Feighner, J., Shrivastava, R., Carman, J., Sramek, J.J., Reines, S.A., Liu, G., Snavely, D., Wyatt-Knowles, E., Hale, J.J., Mills, S.G., MacCoss, M., Swain, C.J., Harrison, T., Hill, R.G., Hefti, F., Scolnick, E.M., Cascieri, M.A., Chicchi, G.G., Sadowski, S., Williams, A.R., Hewson, L., Smith, D., Carlson, E.J., Hargreaves, R.J., Rupniak, N.M.J., 1998. Distinct mechanism for antidepressant activity by blockade of central substance P receptors. Science 281, 1640–1645.

Kramer, M.S., Winokur, A., Kelsey, J., Preskorn, S.H., Rothschild, A.J., Snavely, D., Ghosh, K., Ball, W.A., Reines, S.A., Munjack, D., Apter, J.T., Cunningham, L., Kling, M., Bari, M., Getson, A., Lee, Y., 2004. Demonstration of the efficacy and safety of a novel substance P (NK(1)) Receptor Antagonist in Major Depression. Neuropsychopharmacology 29, 385–392.

Le Poul, E., Laaris, N., Doucet, E., Laporte, A.M., Hamon, M., Lanfumey, L., 1995. Early desensitization of somato-dendritic 5-HT_{1A} autoreceptors in rats treated with fluoxetine or paroxetine. Naunyn-Schmiedeberg's Arch. Pharmacol. 352, 141–148.

Le Poul, E., Laaris, N., Hamon, M., Lanfumey, L., 1997. Fluoxetine-induced desensitization of somatodendritic 5-HT_{1A} autoreceptors is independent of glucocorticoid(s). Synapse 27, 303–312.

Le Poul, E., Boni, C., Hanoun, N., Laporte, A.-M., Laaris, N., Chauveau, J., Hamon, M., Lanfumey, L., 2000. Differential adaptation of brain 5-HT_{1A} and 5-HT_{1B} receptors and 5-HT transporter in rats treated chronically with fluoxetine. Neuropharmacology 39, 110–122.

Malagié, I., Trillat, A.-C., Bourin, M., Jacquot, C., Hen, R., Gardier, A.M., 2001. 5-HT_{1B} autoreceptors limit the effects of selective serotonin reuptake inhibitors in mouse hippocampus but not frontal cortex. J. Neurochem. 76, 865–871.

Parson, L., Justice, J.B., 1994. Quantitative approaches to in vivo brain microdialysis. Crit. Rev. Neurobiol. 8, 189–220.

Ramboz, S., Oosting, R., Amara, D.A., Kung, H.F., Blier, P., Mendelsohn, M., Mann, J.J., Brunner, D., Hen, R., 1998. Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. Proc. Natl. Acad. Sci. U. S. A. 95, 14476–14481.

Rupniak, N.M., 2002. New insights into the antidepressant actions of substance P (NK_1 receptor) antagonists. Can. J. Physiol. Pharm. 80, 489–494.

Shippenberg, T.S., Hen, R., He, M., 2000. Region-specific enhancement of basal extracellular and cocaine-evoked dopamine levels following constitutive deletion of the Serotonin(1B) receptor. J. Neurochem. 75, 258–265.

Siebert, P.D., Larrick, J.W., 1992. Competitive PCR. Nature 359, 557–558.

Spurlock, G., Buckland, P., O'Donovan, M., McGuffin, P., 1994. Lack of effect of antidepressant drugs on the levels of mRNAs encoding serotonergic receptors, synthetic enzymes and 5HT transporter. Neuropharmacology 33, 433–440.

Thompson, A.C., Zapata, A., Justice Jr., J.B., Vaughan, R.A., Sharpe, L.G., Shippenberg, T.S., 2000. Kappa-opioid receptor activation modifies dopamine uptake in the nucleus accumbens and opposes the effects of cocaine. J. Neurosci. 20, 9333–9340.

Toth, M., 2003. 5-HT_{1A} receptor knockout mouse as a genetic model of anxiety. Eur. J. Pharmacol. 463, 177–184.