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Effect of Antidepressant Drugs in Mice Lacking the Norepinephrine Transporter

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One of the main theories concerning the mechanism of action of antidepressant drugs (ADs) is based on the notion that the neurochemical background of depression involves an impairment of central noradrenergic transmission with a concomitant decrease of the norepinephrine (NE) in the synaptic gap. Many ADs increase synaptic NE availability by inhibition of the reuptake of NE. Using mice lacking NE transporter ($NET^{-/-}$) we examined their baseline phenotype as well as the response in the forced swim test (FST) and in the tail suspension test (TST) upon treatment with ADs that display different pharmacological profiles. In both tests, the $NET^{-/-}$ mice behaved like wild-type (WT) mice acutely treated with ADs. Autoradiographic studies showed decreased binding of the β-adrenergic ligand [^{3}H]CGP12177 in the cerebral cortex of $NET^{-/-}$ mice, indicating the changes at the level of β-adrenergic receptors similar to those obtained with ADs treatment. The binding of [^{3}H]prazosin to α_1-adrenergic receptors in the cerebral cortex of $NET^{-/-}$ mice was also decreased, most probably as an adaptive response to the sustained elevation of extracellular NE levels observed in these mice. A pronounced NET knockout-induced shortening of the immobility time in the TST (by ca 50%) compared to WT mice was not reduced any further by NET-inhibiting ADs such as reboxetine, desipramine, and imipramine. Citalopram, which is devoid of affinity for the NET, exerted a significant reduction of immobility time in the $NET^{-/-}$ mice. In the FST, reboxetine, desipramine, imipramine, and citalopram administered acutely did not reduce any further the immobility time shortened by NET knockout itself (ca 25%); however, antidepressant-like action of repeatedly (7 days) administered desipramine was observed in $NET^{-/-}$ mice, indicating that the chronic presence of this drug may also affect other neurochemical targets involved in the behavioral reactions monitored by this test. From the present study, it may be concluded that mice lacking the NET may represent a good model of some aspects of depression-resistant behavior, paralleled with alterations in the expression of adrenergic receptors, which result as an adaptation to elevated levels of extracellular NE.

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INTRODUCTION

The mechanism of action of antidepressant drugs (ADs) has not yet been fully understood. One of the main theories is based on the notion that the neurochemical background of depression involves an impairment of central noradrenergic transmission with a concomitant decrease of the norepinephrine (NE) concentration in the synaptic gap. The noradrenergic hypothesis in depression is supported by the fact that drugs that deplete central nervous system (CNS) monoamines (eg reserpine) can produce depression, and that many antidepressants increase synaptic NE availability by inhibition of the reuptake of NE (Manji *et al*, 2003; Vetulani and Nalepa, 2000). There is also considerable evidence for alterations in growth hormone response to the noradrenergic agonist clonidine in depression (Schatzberg and Schildkraut, 1995; Siever *et al*, 1992). Therefore, it has been widely accepted that CNS noradrenergic neurotransmission has a major impact on the symptomatology of depressive illness as well in the mechanism of action of ADs.

The NE transporter (NET) located in the plasma membrane of presynaptic noradrenergic nerve terminals is responsible for the rapid reuptake of released NE, that is, the NET regulates the extracellular concentration of this amine. The NET is a 12-transmembrane protein and a member of the Na/Cl-dependent monoamine transporters, which also include the transporters for dopamine (DAT) and serotonin (5-hydroxytryptamine, 5-HTT). The gene of the human NET (hNET) is localized on the long arm of

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chromosome 16, and its coding region comprises 14 exons (Pörzgen *et al*, 1995). Tricyclic antidepressants such as desipramine enhance noradrenergic transmission by binding to the NET and blocking its activity (Bönisch and Brüss, 1994); this blockade results in a prolongation of the action of NE in the synapse (Barker and Blakely, 1995). The NET gene is regarded as a candidate gene for major depressive disorders. In previous studies, no association has been found between susceptibility to major depression and a non-synonymous (Owen *et al*, 1999) or five synonymous single nucleotide polymorphisms (Stöber *et al*, 1996) in the gene encoding the hNET. However, more recently, a positive association between a T182C polymorphism in the NET gene and susceptibility to major depressive disorder has been reported in a Japanese population (Inoue *et al*, 2004). Using brain tissues collected post mortem from patients diagnosed with major depression, Klimek *et al* (1997) have shown reduced expression of the NET (decreased [^{3}H]nisoxetine binding) in the locus coeruleus as compared to age-matched normal control subjects. These data as well as differential region-specific effects on the NET of repeated administration of ADs (Herbert *et al*, 2001) have been interpreted as reflecting a compensatory downregulation of the NET in response to an insufficient availability of its substrate NE at the synapse. As preclinical studies also have shown ADs to cause a reduction in NET inhibitor binding in rats and in cultured cells (Bauer and Tejani-Butt, 1992; Zhu and Ordway, 1997), a reduced binding of the selective NET inhibitor [^{3}H]nisoxetine could represent an adaptive response to AD treatment (even if the patients ceased ADs intake some days before death). However, it can also not be ruled out that this finding is owing to an artifact, as recently it was shown that ADs such as desipramine remain very strongly bound to the NET even after prolonged washing (Ordway *et al*, 2005).

On the other hand, Delgado and co-workers have suggested that although both NE and serotonin (5-HT) mediate antidepressant responses, these actions are independent, that is, the depletion of one neurotransmitter had little effect on patients treated with an AD acting primarily on the other neurotransmitter (Delgado and Moreno, 2000; Heninger *et al*, 1996). Therefore, it was interesting to use the recently generated (Xu *et al*, 2000), genetically modified mice lacking the NET (NET$^{-/-}$ mice) for studying the role of the NET and the noradrenergic system in the actions of ADs, and to compare the effects of ADs with those described in previous studies carried out with mice lacking the 5-HT transporter (Holmes *et al*, 2002).

The behavioral efficacy of ADs is usually demonstrated using two common models of stress-induced behavioral responses, the forced swim test (FST) and the tail suspension test (TST). In both experimental paradigms, the animal is subjected to an inescapable stress and typically responds with alternating bouts of escape-oriented behavior and immobility. All major classes of ADs effectively reduce immobility in both tests, confirming their validity as drug-screening paradigms (Borsini and Meli, 1988; Cryan *et al*, 2005a; Petit-Demouliere *et al*, 2005; Porsolt, 2000; Steru *et al*, 1985). Drugs in both experimental paradigms are typically administered acutely. However, some studies have shown that subchronic or acute effects were increased by chronic pre-administration of the AD (Borsini and Meli, 1988; Conti *et al*, 2002; Dulawa *et al*, 2004).

Using NET$^{-/-}$ mice and WT mice, an aim of the present study was to compare the two behavioral tests (FST and TST) and to examine the responsiveness (in the FST) upon acute (single dose) and chronic (7 days) treatment with ADs that display different pharmacological profiles: reboxetine, desipramine, imipramine, and citalopram. Among these drugs, reboxetine is a selective NET inhibitor (Wong *et al*, 2000), the tricyclic compounds imipramine and desipramine are inhibitors of either the NET (desipramine) or the NET and the 5-HTT (imipramine) (Frazer, 1997), and citalopram is a selective 5-HTT inhibitor (SSRI; Hyttel, 1977).

Long-term treatment with ADs is known to cause downregulation of β-adrenergic receptors (Vetulani and Nalepa, 2000), and a role of α_1-adrenergic receptors in some symptoms of depressive illness and in the mechanism of action of ADs has been postulated (Stone and Quartermain, 1999). As it has been demonstrated (Wang *et al*, 1999; Xu *et al*, 2000) that NET$^{-/-}$ mice have elevated extracellular NE concentrations in spite of reduced intraneuronal NE levels, we also examined whether the NET$^{-/-}$ mice show changes in the expression level of cortical β- and α_1-adrenergic receptors.

METHODS

Subjects

Heterozygous NET$^{+/-}$ mice (C57BL/6J background) were mated to produce homozygous NET$^{+/+}$ (WT) and NET$^{-/-}$ mice as previously described by Xu *et al* (2000). For the experiments, we used age-matched adult (ca 3 months) littermates. We used both sexes, as preliminary behavioral experiments revealed no significant sex difference in their responses.

The genotypes were confirmed by PCR and usage of the primers mNATEx2s (5′-GCT TTA TGG CAT GTA GTG TGC AC-3′), mNATEx2as (5′-GCT TTC TGC TTG AAC TTG AAG GC-3′), and EGFPas (5′-GCC GGA CAC GCT GAA CTT GTG-3′) to amplify a 700 and 500 bp PCR product in case of WT and NET$^{-/-}$ mice, respectively.

The animals had free access to food and water and were kept at a constant room temperature (24°C), under 12-h light/dark cycle (light on at 06:00). Animals were kept according to the guidelines of the European Union (86/609/EWG).

α_1- and β-Adrenergic Receptor Autoradiography

Quantitative autoradiography was carried out as described by Nalepa *et al* (2005) and Strazielle *et al* (1999). After decapitation, mice brains were rapidly removed, frozen, and stored on dry ice. Consecutive coronal sections (12 μm) were cut at −23°C using a Jung CM 3000 cryostat (Leica, Germany). Experiments were evaluated in the coronal sections (Paxinos and Franklin, 2001).

The slices were sectioned and thaw-mounted on gelatin-coated glass microscope slides. They were stored at −70°C. Immediately before use, the slide-mounted sections were dried at room temperature.

For α_1-adrenoceptor binding, sections of both genotypes were thawed and preincubated for 1 h at room temperature in a Krebs modified buffer containing 10 mM Na_2HPO_4 (pH 7.8), 119 mM NaCl, 6 mM KCl, 1.2 mM $MgSO_4$, and 1.3 mM $CaCl_2$. The sections were then further incubated for 1 h in the same buffer containing 0.9 nM of [^{3}H]prazosin (specific activity: 77 Ci/mmol; Du Pont). Thereafter, they were washed in the same buffer two times (2 min, ice-cold). Nonspecific binding was determined by incubating parallel sections in the presence of 40 μM phentolamine mesylate (Research Biochemicals). For β-adrenergic receptor binding, the incubations were performed at 25°C for 60 min in 50 mM Tris-HCl buffer (pH 7.4) containing 120 mM NaCl and 5 mM KCl with 4 nM of the β-adrenergic ligand [^{3}H]CGP12177 (specific activity: 42.5 Ci/mmol; Du Pont). Propranolol (5 μM; Sigma) was used to determine nonspecific labeling.

Dried tissue sections were exposed for 5 days to tritium-sensitive screens (FujiFilm, Düsseldorf, Germany) along with [^{3}H]microscales (Amersham) as standard. The images were obtained by means of a FujiFilm BAS 5000 Phosphorimager. They were analyzed using FujiFilm software (Image Gauge, Version 4.0) and quantified by computer-generated curves derived from the standards. The pixels of images from sections showing nonspecific binding were subtracted from the images of adjacent sections with total binding. The results are expressed as fmol of bound radioligand per mg protein.

The identification and nomenclature of brain structures is based on the mouse brain atlas of Paxinos and Franklin (2001).

Locomotor Activity

The locomotor activity was measured in custom-made circular aluminum photoresistor actometers (cages with walls, 10 cm in height, 30 cm in diameter, equipped with two light sources and two photoresistors, arranged so that the beams crossed the center) in which mice were placed individually. The construction of the apparatus allowed collection of only the gross movement data. Experiments were carried out during the light phase. The measurements of locomotor activity lasted for 30 min.

Drugs and Drug Treatment

Reboxetine HCl was obtained from Pharmacia & Upjohn (Kalamazoo, MI, USA); desipramine HCl and imipramine HCl were from Sigma/RBI; citalopram HBr was kindly provided by Lundbeck.

Drug doses were 20 mg/kg for reboxetine and desipramine, 25 mg/kg for imipramine, and 10 mg/kg for citalopram. Doses were chosen on the basis of previous reports in mice of the anti-immobility effects of reboxetine (Wong *et al*, 2000), desipramine (Lucki *et al*, 2001; Wong *et al*, 2000), imipramine (Bourin *et al*, 1998; Liu and Geshenfeld, 2003; Wong *et al*, 2000), and citalopram (Cryan *et al*, 2004; Perrault *et al*, 1992).

Drugs were dissolved in 0.9% physiological saline (desipramine and citalopram) or in distilled water (reboxetine and imipramine). Injections were given intraperitoneally (i.p.) in a volume of 10 ml/kg body weight, either acutely (single dose) or repeatedly (once daily for 7 days). Control animals received an appropriate vehicle. In the FST experiments where repeated treatment with antidepressant was studied, all animals received seven i.p. daily injections of either vehicle or the drug. In the acutely treated groups, the animals received for 6 days a daily i.p. injection of vehicle, and on day 7 a single dose of the AD.

All behavioral testing was conducted with the use of separate sets of mice, different for each drug and test, that is, each mouse was subjected to only one test session.

Tail Suspension Test

For the TST (Steru *et al*, 1985), mice were securely fastened by the distal end of the tail to a flat surface and suspended. The presence or absence of immobility, defined as the absence of limb movement, was assessed over a 6-min session by a highly experienced observer who was not aware of the genotype. Antidepressants were administered 30–40 min before testing.

Forced Swim Test

For the FST, mice were placed in a transparent cylinder (20 cm in diameter) filled with water (23–25°C). Filling the cylinder to a depth of 12 cm prevented mice from using their tails to support themselves in the water. Immobility was defined as a cessation of limb movements except minor movement necessary to keep the mouse afloat. Immobility was measured during the last 4 min of a 6-min session by a highly experienced observer who was not aware of the genotype. The test was performed 1 h after administration of the last dose of the drug (or vehicle).

Statistical Analysis

Analysis of the data obtained from locomotor activity measurements, as well as the biochemical data (autoradiograms), was made using the Student's test to compare the genotype response (WT *vs* NET$^{-/-}$). Similar analysis was used to compare the response of drug-free mice of the two genotypes in the TST and FST. The effects of antidepressants in the TST and FST in a given genotype were analyzed using two-way ANOVA followed by Bonferroni post-test.

RESULTS

Autoradiography of α_1- and β-Adrenergic Receptors in the Cerebral Cortex of WT and NET$^{-/-}$ Mice

Binding of [^{3}H]prazosin to cortical α_1-adrenergic receptors was characterized by higher levels in the superficial layer I than in cingulate cortex, and in bands corresponding to layers III and V (Figure 1a). In all of these regions, a significant decrease in [^{3}H]prazosin binding was observed in NET$^{-/-}$ mice, as compared to WT animals (Figure 1b).

Cortical β-adrenergic receptors labeled with [^{3}H]CGP12177 were rather homogenously distributed in the different areas of the cortex. However, average binding was higher in the superficial as compared to deep layers of the cortex (Figure 2a). Significant downregulation of [^{3}H]CGP12177 binding was observed in all cortical regions

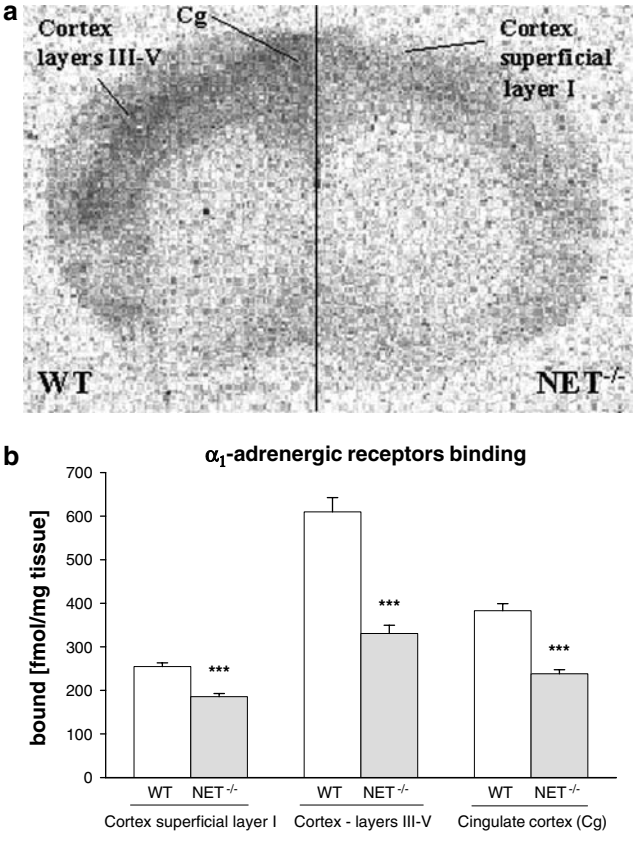

Figure 1 (a) Representative autoradiogram of [^{3}H]prazosin binding to α_1-adrenergic receptors in coronal sections of a control wild-type (WT) and NET$^{-/-}$ mouse. The brain regions used for quantitative analysis were chosen according to Paxinos and Franklin (2001). (b) Effect of genotype on the binding of [^{3}H]prazosin to α_1-adrenergic receptors in the mouse brain cortex. Data represent the mean ± SEM, $n = 7$ (fmol/mg tissue). Student's test (two-tailed) was used to compare groups WT *vs* NET$^{-/-}$, ***$p < 0.001$.

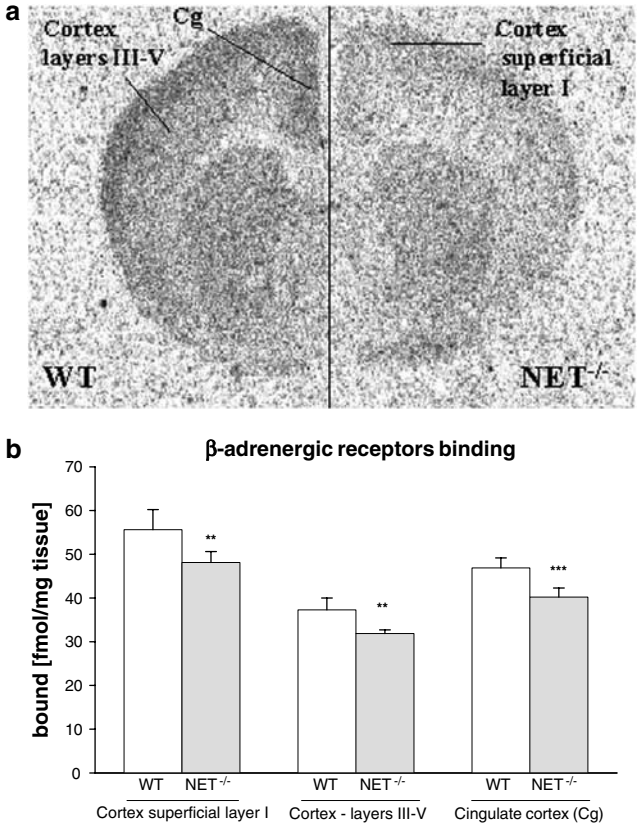

Figure 2 (a) Representative autoradiogram of [^{3}H]CGP 12177 binding to β-adrenergic receptors in coronal sections of a control WT and NET$^{-/-}$ mouse. The brain regions used for quantitative analysis were chosen according to Paxinos and Franklin (2001). (b) Effect of genotype on the binding of [^{3}H]CGP 1277 to β-adrenergic receptors in the mouse brain cortex. Data represent the mean ± SEM, $n = 7$ (fmol/mg tissue). Student's test (two-tailed) was used to compare groups WT *vs* NET$^{-/-}$, **$p < 0.01$, ***$p < 0.001$.

of NET$^{-/-}$ mice as compared to WT animals (Figure 2b). Substantial binding of this ligand was also observed in the caudate-putamen (Figure 2a); however, no significant differences were noted between the genotypes (data not shown).

Comparison of WT and NET$^{-/-}$ Mice in Behavioral Experiments

As shown in Figure 3, locomotor activity of untreated NET$^{-/-}$ mice, measured in photoresistor actometers, was significantly lower (ca 30% reduction) as compared to the control WT mice. In the FST, on the other hand, untreated NET$^{-/-}$ mice displayed significantly shorter (by 25–30%) immobility time than WT animals (Figure 5). Furthermore, in the TST, the shortening of the immobility time in untreated NET$^{-/-}$ mice was even more pronounced (ca 45% reduction) compared to WT mice (Figure 4).

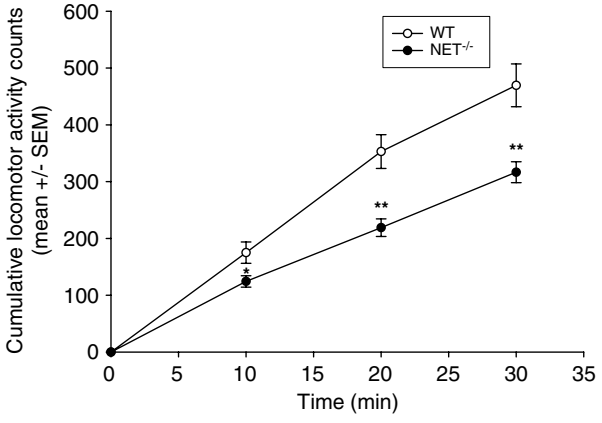

Figure 3 Basal locomotor activity (photoresistor actometers, two light beams) in WT and NET$^{-/-}$ mice. Locomotor activity was measured for 30 min at 10-min intervals. The results are means ± SEM, $n = 14$ mice per group. The statistical significance was calculated using one-way ANOVA, followed by Dunnett's test *$p < 0.05$; **$p < 0.001$ *vs* WT.

Tail Suspension Test

Concerning the effects of reboxetine in the TST, there was a significant effect of genotype ($F_{1,71} = 55.9$; $p < 0.0001$), drug ($F_{1,71} = 11.53$; $p = 0.011$), and genotype × drug interaction ($F_{1,71} = 9.64$; $p = 0.0027$) on immobility time. As shown in Figure 4a, reboxetine significantly shortened the immobility time in WT mice ($p < 0.001$), but had no effect in NET$^{-/-}$

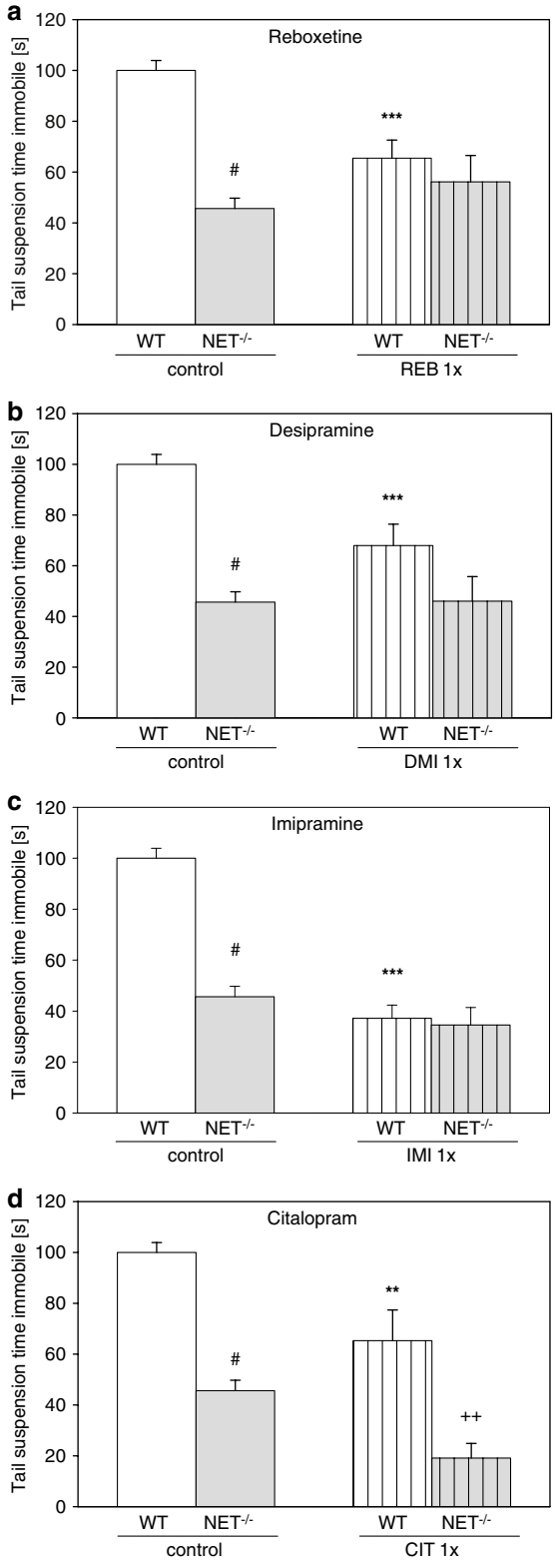

Figure 4 Responses of WT and NET$^{-/-}$ mice in the TST following acute (1 ×) administration of (a) reboxetine (REB, 20 mg/kg i.p.), (b) desipramine (DMI, 20 mg/kg i.p.), (c) imipramine (IMI, 25 mg/kg i.p.), and (d) citalopram (CIT, 10 mg/kg i.p.). The results are means ± SEM, $n = 16$–18 mice per group. The statistical significance was calculated using two-way ANOVA, followed by Bonferroni post-test and *t*-test for comparison of untreated (*control*) NET$^{-/-}$ *vs* WT mice. $^{\#}p < 0.001$ NET$^{-/-}$ *vs* WT *control*; $^{**}p < 0.01$; $^{***}p < 0.001$ *vs* WT *control*; $^{++}p < 0.01$ *vs* NET$^{-/-}$ *control*.

mice. For the effect of desipramine in the TST, there was a significant effect of genotype ($F_{1,68} = 41.96$; $p < 0.0001$), drug ($F_{1,68} = 6.29$; $p = 0.0146$), and genotype × drug interaction ($F_{1,68} = 7.83$; $p = 0.0067$). As shown in Figure 4b, desipramine significantly reduced the immobility time in WT mice ($p < 0.001$), but had no effect in NET$^{-/-}$ mice. For the effect of imipramine in this test, there was a significant effect of genotype ($F_{1,68} = 31.24$; $p < 0.0001$), drug ($F_{1,68} = 46.85$; $p < 0.0001$), and genotype × drug interaction ($F_{1,68} = 24.92$; $p < 0.0001$). As shown in Figure 4c, imipramine significantly shortened the immobility time in WT mice ($p < 0.001$), but had no effect in NET$^{-/-}$ mice. On the other hand, for citalopram, there was a significant effect of genotype ($F_{1,61} = 70.46$; $p < 0.0001$), drug ($F_{1,61} = 25.21$; $p < 0.0001$), but no genotype × drug interaction ($F_{1,61} = 0.12$; $p = 0.7284$). As shown in Figure 4d, citalopram significantly reduced the immobility time in WT mice ($p < 0.01$) and also in NET$^{-/-}$ mice ($p < 0.01$).

Forced Swim Test

In the FST, the statistical analysis revealed that reboxetine induced a significant effect of genotype ($F_{1,54} = 28.01$; $p < 0.0001$), drug ($F_{2,54} = 6.98$; $p = 0.002$) but no genotype × drug interaction ($F_{2,54} = 2.16$; $p = 0.1255$) on immobility time. As shown in Figure 5a, reboxetine significantly shortened the immobility time in WT mice given either acutely ($p < 0.001$) or repeatedly ($p < 0.001$). The drug had no effect in NET$^{-/-}$ mice given acutely or repeatedly. For desipramine in the FST, there was a significant effect of genotype ($F_{1,42} = 23.73$; $p < 0.0001$), drug ($F_{2,42} = 9.75$; $p = 0.0003$) but no genotype × drug interaction ($F_{2,42} = 0.22$; $p = 0.8053$) on immobility time. As shown in Figure 5b, desipramine significantly reduced the immobility time in WT mice given either acutely ($p < 0.05$) or repeatedly ($p < 0.01$). In NET$^{-/-}$ mice, the drug given acutely had no effect, but given repeatedly significantly reduced immobility time ($p < 0.01$). For imipramine in the FST, there was significant genotype × drug interaction ($F_{2,59} = 4.68$; $p = 0.0129$) on immobility time. Figure 5c shows the effect of imipramine, which significantly reduced the immobility time in WT mice given either acutely ($p < 0.01$) or repeatedly ($p < 0.05$), but had no effect in NET$^{-/-}$ mice. For the effect of citalopram in the FST, there was a significant genotype × drug interaction ($F_{2,49} = 4.62$; $p = 0.0146$) on immobility time. As shown in Figure 5d, citalopram had no effect in WT mice given acutely or repeatedly and also in NET$^{-/-}$ mice given acutely. After repeated administration, it significantly increased immobility time in NET$^{-/-}$ mice ($p < 0.05$).

DISCUSSION

The present study shows that NET$^{-/-}$ mice display a baseline phenotype of reduced immobility in both tests employed, the FST and TST, that is, the NET$^{-/-}$ mice behaved like WT mice acutely treated with ADs. These findings confirm the results of Xu *et al* (2000) and show that inactivation of the NET gene mimics the effects of ADs in behavioral tests. The behavioral effects were significant, repeatable, and intuitively expected in the light of what has

been known about the role of NE in the mechanism of action of ADs, at least those that inhibit the NET.

Our autoradiographic studies showed decreased binding of the β-adrenergic ligand [^{3}H]CGP12177 in the cerebral cortex of NET$^{-/-}$ mice, indicating downregulation of β-adrenergic receptors. This result also indicates that knockout of the NET results in biochemical changes similar to those obtained with ADs treatment, as many studies consistently have shown antidepressant-induced downregulation of these receptors (for review, see Vetulani and Nalepa, 2000). However, it must be added that clinically active selective serotonin reuptake inhibitors administered repeatedly do not induce downregulation of β-adrenergic receptors. Nevertheless, the adaptive changes in the noradrenergic system were considered as an important part of antidepressant treatment, although results concerning changes in α_1-adrenergic receptors were often contradictory.

In the present study, we show that binding of [^{3}H]prazosin to α_1-adrenergic receptors was significantly reduced in the cerebral cortex of NET$^{-/-}$ mice. These data agree with those obtained by Xu *et al* (2000), who have demonstrated a significant decrease in hippocampal α_1-adrenoceptor binding in the NET$^{-/-}$ mice. These adaptive changes at the level of central adrenergic receptors might be interpreted as a response to sustained elevated levels of extracellular NE in the NET$^{-/-}$ mice, which has indeed been shown by Wang *et al* (1999) and Xu *et al* (2000). In many studies, it has been reported that repeated treatment with tricyclic ADs or electroconvulsive shock increases either the density or agonist affinity of brain α_1-adrenergic receptors (Menkes and Aghajanian, 1981; Menkes *et al*, 1980; Nalepa *et al*, 2002; Vetulani *et al*, 1984). However, not all investigators have been able to confirm the above findings (Heal, 1984; Stockmeier *et al*, 1987; Hayakawa *et al*, 1992), indicating that variables involved have not been completely isolated.

The NET$^{-/-}$ mice were less immobile in the FST and TST, despite lower basal locomotor activity. However, even if a sedative effect is observed in the locomotor-activity measuring device, antidepressant-like activity may be perceived in the FST (David *et al*, 2003; Malinge *et al*, 1988). The observed downregulation of α_1-adrenoceptor in NET$^{-/-}$ mice together with their increased activity in the behavioral tests is an interesting finding, as blockade of α_1-adrenoceptors by prazosin (Stone and Quartermain, 1999) or terazosin (Stone *et al*, 2003) has been reported to produce cessation of behavioral activity, also in the TST.

Among many mutant mice lines generated, only a few can be regarded as simple genetic depression models or as useful models for elucidating the mechanism of action of antidepressants (Cryan and Mombereau, 2004; Urani *et al*, 2005). An example illustrating this notion are the data provided by Holmes *et al* (2002), who have studied antidepressant responses in 5-HTT knockout mice (5-HTT$^{-/-}$), and by Cryan *et al* (2004), who have examined

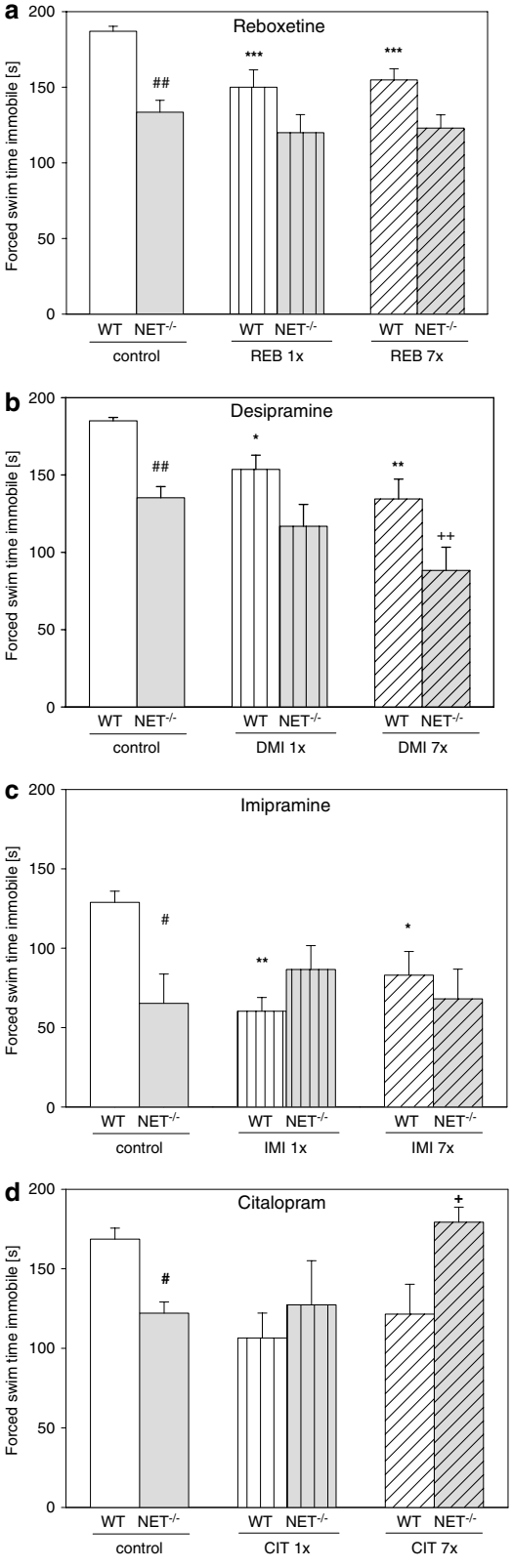

Figure 5 Responses of WT and NET$^{-/-}$ mice in the FST following acute (1 ×) and repeated (7 ×) administration of (a) reboxetine (REB, 20 mg/kg i.p.), (b) desipramine (DMI, 20 mg/kg i.p.), (c) imipramine (IMI, 25 mg/kg i.p.), and (d) citalopram (CIT, 10 mg/kg i.p.). The results are means ± SEM, $n = 10$–12 mice per group. The statistical significance was calculated using two-way ANOVA, followed by Bonferroni post-test and *t*-test for comparison of untreated (*control*) NET$^{-/-}$ *vs* WT mice. $^{\#}p<0.01$; $^{\#\#}p<0.001$ NET$^{-/-}$ *vs* WT *control*; $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$ *vs* WT *control*; $^{+}p<0.05$; $^{++}p<0.01$ *vs* NET$^{-/-}$ *control*.

dopamine β-hydroxylase knockout ($Dbh^{-/-}$) mice, which are unable to synthesize NE and epinephrine. In these two animal models, no differences in baseline immobility were observed in the knockout mice with a C57BL/6 background. Therefore, these authors concluded that NE and 5-HT are not necessary for the tonic regulation of baseline performance in the tasks involved in both behavioral tests. However, NE and 5-HT appear to be necessary for the reduction of immobility in these tasks when their levels are increased by ADs. In fact, the drugs used in the above-mentioned studies exerted rather expected effects. Anti-immobility effects of the 5-HT reuptake inhibitor fluoxetine were abolished in $5\text{-}HTT^{-/-}$ mice, whereas these mice retained sensitivity to the anti-immobility effects of the NE reuptake inhibitor desipramine and the mixed 5-HT/NE reuptake inhibitor imipramine (Holmes *et al*, 2002). Similarly, $Dbh^{-/-}$ mice failed to respond to the behavioral effects of the NET inhibitors desipramine and reboxetine, but effects of SSRIs (fluoxetine, sertraline, and paroxetine, but not citalopram) were also absent or severely attenuated in these mice (Cryan *et al*, 2004). These data indicate that serotonin could underlie effects that are modified by changes in NE function.

In the present study, we observed a pronounced NET knockout-induced shortening of the immobility time in the TST by about 50% compared to WT mice, and NET-inhibiting antidepressants such as reboxetine, desipramine, and imipramine caused no further reduction. However, in $NET^{-/-}$ mice, imipramine showed a tendency (not significant) toward shortening of the immobility time, possibly because of its additional property as 5-HTT inhibitor (Frazer, 1997). On the other hand, citalopram, which is devoid of affinity for the NET, exerted a significant reduction of immobility time in the $NET^{-/-}$ mice. Citalopram is the most selective 5-HTT inhibitor among the SSRIs, with an 'affinity' for the 5-HTT of about 14 nM (Hyttel, 1977). Therefore, its efficacy in the $NET^{-/-}$ mice may not be impacted by alterations to the NE transmitter system.

Although rodent behavioral models have a good predictive validity for ADs and they are sensitive to the acute administration of these compounds, it is widely recognized that the symptoms of depression in patients are only ameliorated after chronic drug treatment. Therefore, we decided to check whether the effects of ADs in the FST are dependent on the duration of drug treatment.

The strain of mice strongly influences the animal response in the FST (Alcaro *et al*, 2002; David *et al*, 2003; Lucki *et al*, 2001), with C57BL/6J mice being moderately responsive to ADs in this test. Some authors have postulated that it is the high brain dopamine level in this strain that is a limiting factor for the antidepressant-like effects of ADs in this test (Renard *et al*, 2004). However, in our hands, WT animals were responsive to the acute and repeated administration of the antidepressants used in the present study, with the exception of citalopram, which was not effective, similar as in the studies reported by David *et al* (2003).

The lack of effect of reboxetine administered acutely or repeatedly in the FST clearly indicates that for the action of this drug, the presence of a functional NET is necessary. Also desipramine and imipramine, given acutely, were not active in the FST in $NET^{-/-}$ mice. However, the antidepressant-like action of repeatedly administered desipramine (but not imipramine) indicates that the chronic presence of this NET inhibitor may affect also other neurochemical targets such as various subtypes of serotonin or dopamine receptors (Vetulani and Nalepa, 2000; Cryan *et al*, 2005b; Petit-Demouliere *et al*, 2005; Renard *et al*, 2001, 2003; Dziedzicka-Wasylewska *et al*, 2000). The lack of effect of repeated treatment of $NET^{-/-}$ mice with imipramine might result from the additional affinity of this drug for the 5-HTT. Such conclusion seems justified regarding the effects of citalopram, discussed below.

Citalopram is unique among the clinically available SSRIs in having the greatest selectivity for the 5-HTT over the NET *in vitro* (Sanchez and Hyttel, 1999). Therefore, repeated administration of this drug mimics in some ways a situation observed in mice deficient in 5-HTT. Holmes *et al* (2002) have shown that $5\text{-}HTT^{-/-}$ mice (on the 129S6 background, however) showed markedly increased immobility in the FST. These mice exhibited reduced neuromuscular strength and stamina on the wire hang test indicating neuromuscular impairment, and this might have seriously compromised swimming in the FST. Also in the present study (using mice with a C57BL/6 background), repeated administration of citalopram significantly increased the immobility time in WT animals, which resembles the effect observed in $5\text{-}HTT^{-/-}$ mice. Presently, we have no explanation for this observation.

Mice lacking the NET may represent a good model of some aspects of depression-resistant behavior, paralleled with alterations in the expression of adrenergic receptors, which result as an adaptation to elevated levels of extracellular NE. In addition to the changes reported in the present studies, the $NET^{-/-}$ mice have been shown to be supersensitive to psychostimulants (Xu *et al*, 2000), a phenomenon that has been also observed following the repeated treatment with antidepressants (Dziedzicka-Wasylewska and Rogoż, 1998; Maj *et al*, 1984; Rogoż and Dziedzicka-Wasylewska, 1999). More biochemical studies are necessary to elucidate further molecular mechanisms underlying these changes. Nevertheless, together with other knockout models, the $NET^{-/-}$ mice provide a useful tool for delineating the mechanisms underlying the depression-resistant behavior, and the pharmacological actions of various classes of antidepressants.

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