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Short communication

Effect of two behavioral tests on corticosterone level in plasma of mice lacking the noradrenaline transporter

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Abstract:
In the present study, we investigated plasma corticosterone levels of genetically modified mice lacking noradrenaline transporter ($NET^{-/-}$), in response to the forced swim test (FST) and tail suspension test (TST). FST strongly increased the plasma corticosterone level in the first minute after the test (significantly only in $NET^{+/+}$ mice), while TST was without any significant effect in both genotypes studied. A single dose of tianeptine (20 mg/kg, *ip*) shortened immobility time in both tests in $NET^{-/-}$ mice, as well as $NET^{+/+}$ mice subjected to FST, but not TST. The lack of effect of tianeptine in control animals ($NET^{+/+}$) subjected to TST is also reflected in unchanged levels of plasma corticosterone.

Key words:
tianeptine, corticosterone, PCPA, NET, knock-out mice, C57BL/6J, TST, FST

Abbreviations: CRF – corticotropin-releasing factor, FST – forced swim test, HPA axis – hypothalamus-pituitary-adrenal axis, NE – noradrenaline, NET – noradrenaline transporter, PCPA – 4-chloro-DL-phenylalanine, TST – tail suspension test

Introduction

The neurochemical hypothesis of depression centers on the impairment of central noradrenergic transmission and the concomitant decrease of noradrenaline (NE) concentration in the synaptic space. Recent development of genetically modified mice lacking the noradrenaline transporter ($NET^{-/-}$) [13] opened new perspectives for studying the role of the noradrenergic system in animal models used in search for the mechanism of action of antidepressants. We have shown previously that these animals display "depressive-resistant" behavior, since they show significantly shorter immobility time in both the forced swim test (FST) and tail suspension test (TST), despite significantly lower locomotor activity measured in photoresistor actometers in comparison to wild-type animals ($NET^{+/+}$) [4].

In the present study, we investigated the plasma level of corticosterone in the $NET^{-/-}$ *vs*. $NET^{+/+}$ mice in response to FST and TST, in order to see whether there are any differences in hypothalamus-pituitary-adrenal (HPA) axis activity. Such genotypic differences might possibly underlie baseline differences in

their behavior as well as in their behavioral response to tianeptine. Tianeptine has been postulated to act as antidepressant by reducing the overactivity of the HPA axis [2] (which is deregulated in some forms of depression). We therefore investigated the effect of this drug on the level of plasma corticosterone in both genotypes after the FST and TST. Tianeptine does not affect the NET directly, but it is regarded as an enhancer of serotonin reuptake [9]. In the present investigation, we used 4-chloro-DL-phenylalanine (PCPA), an inhibitor of serotonin synthesis [3], in order to check whether the level of endogenous serotonin affected the response of mice to tianeptine.

Materials and Methods

Animals

Heterozygous NET$^{+/-}$ mice (C57BL/6J background), obtained from Dr. M. Caron (Duke University, Medical Center, Durham, NC, USA), were mated to produce homozygous NET$^{+/+}$ and NET$^{-/-}$ mice as described [13]. For the experiments, we used age-matched adult (3–5 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 mNETEx2s (5'-GCT TTA TGG CAT GTA GTG TGC AC-3'), mNETEx2as (5'-GCT TTC TGC TTG AAC TTG AAG GC-3'), and EGFPas (5'-GCC GGA CAC GCTGAA CTT GTG-3') to amplify a 700 and 500 bp PCR product in the case of NET$^{+/+}$ 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. Animals were kept according to the guidelines of the European Union (86/609/EWG).

Drugs and drug treatment

Tianeptine sodium salt and 4-chloro-DL-phenylalanine (PCPA) were from Sigma, USA. The tianeptine dose was 20 mg/kg and was chosen on the basis of our previous study (data not shown). Tianeptine was dissolved in water. The 4-chloro-DL-phenylalanine (PCPA) dose was 300 mg/kg (chosen from previous reports [3]) and was suspended in a 1% aqueous solution of Tween 80. PCPA was administered intraperitoneally (*ip*) once a day for four consecutive days, and tianeptine was administered *ip* on the last day, 15 min after the last PCPA injection. Thirty minutes later, the FST was carried out.

Locomotor activity

Locomotor activity was measured in actometers (OPTO-M3), in which mice were individually placed. The apparatus design allowed collection of the ambulation data; ambulation was measured 45 min after the last PCPA injection. The measurements of locomotor activity lasted for 30 min. Experiments were carried out during the light phase.

Tail suspension test

For the TST [11], 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. Tianeptine was administered 30 min before testing.

Forced swim test

For the FST, mice were placed in the 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 for minor movements 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 [10]. The test was performed 30 min after administration of the last dose of the drug (or vehicle).

Radioimmunoassay

The trunk blood was collected, EDTA was added, and samples were centrifuged at 800 × g for 15 min; plasma was removed and stored at –20°C until assay. Corticosterone was extracted from the plasma, added to ethanol, and measured by a radioimmunological method. 1,2,6,7-[^{3}H]-corticosterone (s.a. 85 Ci/mmol)

was purchased from the Radiochemical Centre, Amersham; an antiserum was obtained from Chemicon. Cross-reactivity of that antiserum with 11-dehydrocorticosterone and deoxycorticosterone was 0.67 and 1.5%, respectively. Cross-reactivity with other steroids was below 0.01%. The assay sensitivity was 10 pg/tube. Intra- and inter-assay coefficients of variation were lower than 5 and 8%, respectively.

Statistical analysis

Data obtained from FST after PCPA administration were subjected to one-way ANOVA followed by Bonferroni *post-hoc* analysis. Student's *t*-test compared corticosterone concentrations between $NET^{+/+}$ and $NET^{-/-}$ after TST and FST. A value of $p < 0.05$ was considered to be significant.

Results and Discussion

As can be seen in Figure 1, the $NET^{-/-}$ mice displayed shorter immobility times in both tests. These measures are the preferred method for screening antidepressant efficacy in rodents. Previous studies [4, 13] have shown that these animals represent a depression-resistant phenotype.

A single dose of tianeptine was able to further shorten the immobility time in $NET^{-/-}$ mice. The drug was also active in control animals subjected to FST, but – surprisingly – it did not affect response to the TST (Fig. 1).

In order to identify possible differences between the tests, we decided to measure the level of corticosterone in plasma from both groups of animals, following behavioral treatment. Mean basal corticosterone levels did not differ among the groups (Fig. 2). Similarly, Haenisch et al. [6] reported no differences in the level of mRNA encoding corticotrophin-releasing factor (CRF) in the hypothalamus of $NET^{-/-}$ mice. However, when each sex was examined separately, female mice of both phenotypes displayed slightly higher levels of plasma corticosterone (the difference reached significance for the $NET^{+/+}$ genotype) (Fig. 2). FST was a much stronger stressor than TST. The increase in plasma corticosterone in the first minute following FST was significantly stronger in the $NET^{+/+}$ mice as compared to $NET^{-/-}$ animals. In contrast, TST did not affect the level of plasma corticosterone in either group of mice (Fig. 2).

Another series of experiments examined the effect of tianeptine on the level of corticosterone following FST. Administration of the drug prior to the behavioral procedure blunted the response in both groups of mice. The effect of tianeptine in decreasing corticosterone levels was significantly stronger in the $NET^{-/-}$ mice

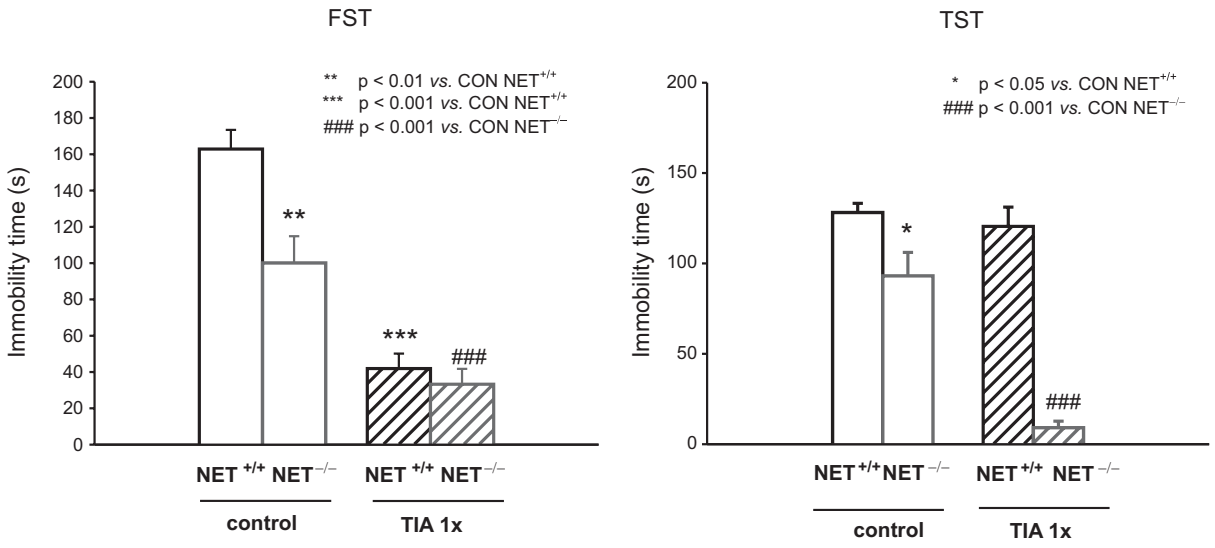

Fig. 1. Effect of tianeptine (TIA) at the dose 20 mg/kg on immobility time in $NET^{+/+}$ and $NET^{-/-}$ mice subjected to the forced swim test (FST) and tail suspension test (TST). Results are the mean immobility time ± SEM. The groups consisted of 10 animals, each treated 30 min prior to the test. Statistical analysis was performed by Student's *t*-test

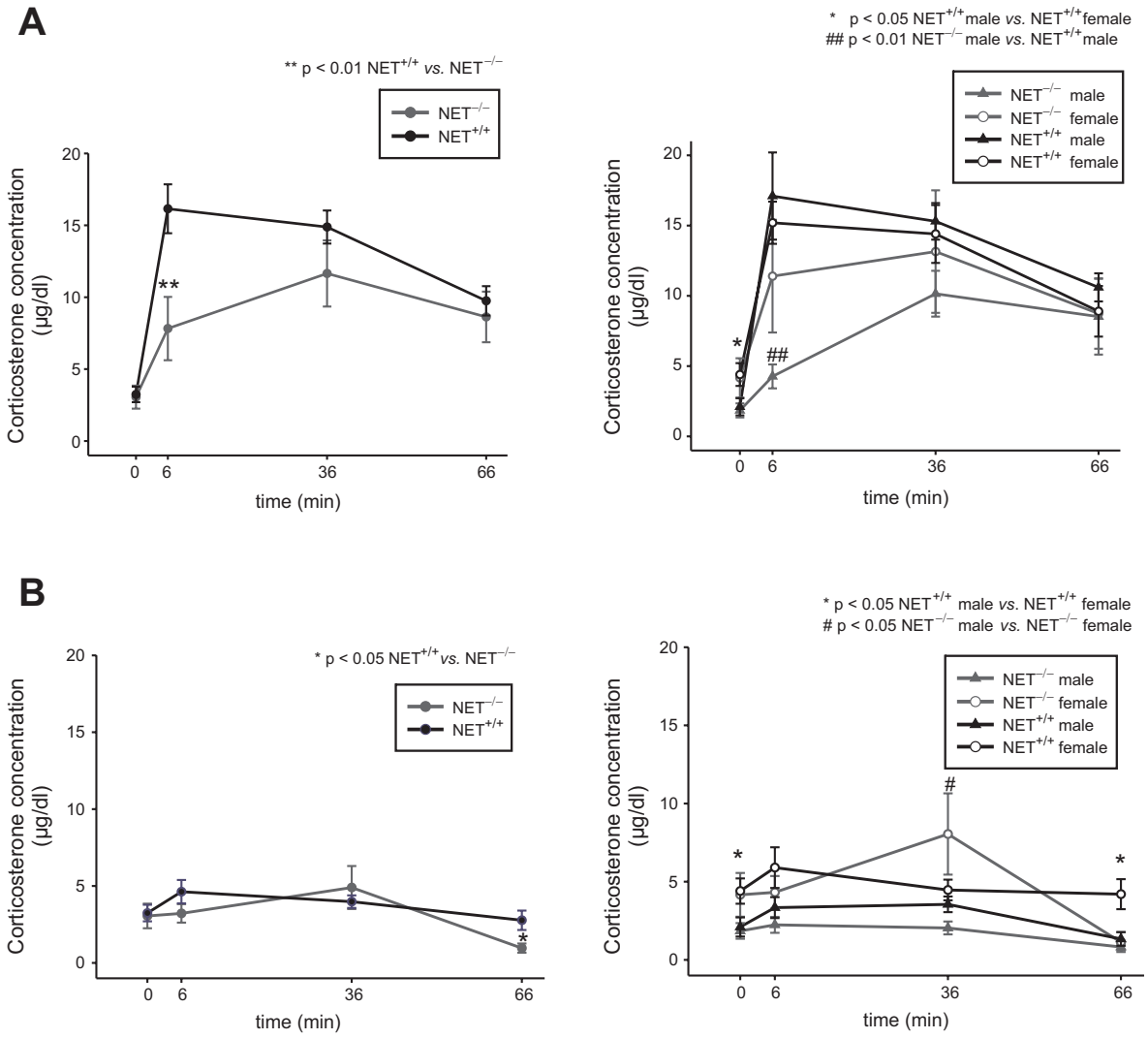

Fig. 2. Corticosterone concentration changes after forced swim test (FST) **(A)** and tail suspension test (TST) **(B)** in NET$^{+/+}$ and NET$^{-/-}$ mice. Results are the mean corticosterone concentration ± SEM. The groups consisted of 16 animals each (8 male and 8 female). The samples were collected 6, 36 and 66 min after the tests. Statistical analysis was performed by Student's *t*-test

(Fig. 3). These data support the postulated role of the HPA axis in the mechanism of action of tianeptine [2].

Tianeptine was not active in NET$^{+/+}$ mice subjected to TST. This could potentially be explained by a much weaker response of these animals to TST, as indicated by corticosterone levels. However, it must be stressed that tianeptine was active in NET$^{-/-}$ animals subjected to TST – as demonstrated by further shortening of immobility time. Tianeptine cannot be linked to the regulation of plasma corticosterone level in these animals, since TST did not influence this index.

We also show how endogenous levels of serotonin affect the response of mice to tianeptine in the FST. In a previous study [4], we have shown that acute citalopram was not active in FST, in either control or NET$^{-/-}$ mice (although it shortened the immobility time during TST in both groups of animals). However, determining the role of serotonin is difficult, since – regardless of the effect of citalopram on serotonergic transmission – one must take into account the differing sensitivity of various mice strains to behavioral procedures [7]. Here, we used the standard procedure of administering PCPA, which has been shown to inhibit serotonin synthesis by blocking tryptophan hydroxylase activity [3].

The results presented in Figure 4 show that pretreatment with PCPA itself reduced the immobility

time in NET$^{+/+}$ animals. A similar effect of PCPA treatment has been reported in rats [5]. These authors postulated an effect of PCPA on general activity; however, in our experiments, we did not observe any changes in locomotor activity following PCPA administration (Fig. 4, inset). Tianeptine also reduced immobility time; concomitant administration of both drugs resulted in a reduction similar to the effect produced by tianeptine alone.

In NET$^{-/-}$ mice, PCPA did not affect immobility time, possibly due to altered functioning of the neuronal network, since the lack of noradrenergic transporters in these animals leads to changes in noradrenaline/serotonin reuptake homeostasis [12]. In NET$^{-/-}$ mice, serotonergic neurons were shown to take up noradrenaline via serotonin transporters and then release it in response to neuronal activity. Therefore, administration of PCPA to these animals was not as effective in influencing the behavioral response as it was in control mice. On the other hand, PCPA administered together with tianeptine to NET$^{-/-}$ animals changed their response to tianeptine; the antidepressant effect was much less pronounced in comparison to control animals.

The results obtained in the present study indicate that FST might be considered a much stronger stressor than TST, since it significantly increased the plasma level of corticosterone in the first minute after the test – while TST was without effect. As Cryan et al. [1] have pointed out, there are different neurobiological substrates underlying the behavioral re-

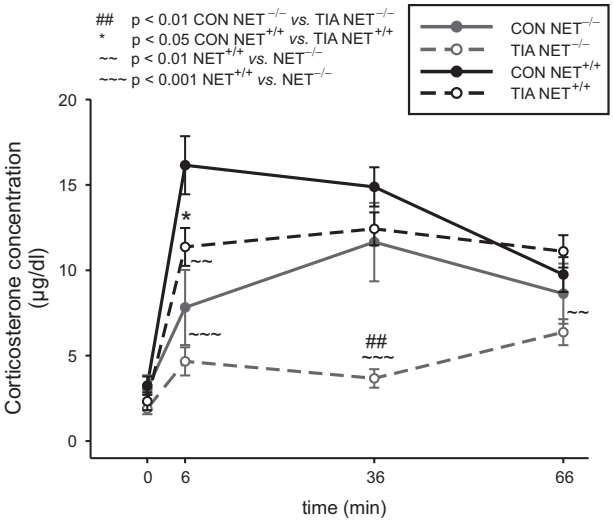

Fig. 3. Effect of tianeptine (TIA, 20 mg/kg) on plasma corticosterone concentration changes in NET$^{+/+}$ and NET$^{-/-}$ mice after forced swim test (FST). Results are the mean corticosterone concentration ± SEM. The groups consisted of 16 animals each (8 male and 8 female). The samples were collected 6, 36 and 66 min after the tests. Statistical analysis was performed by Student's *t*-test

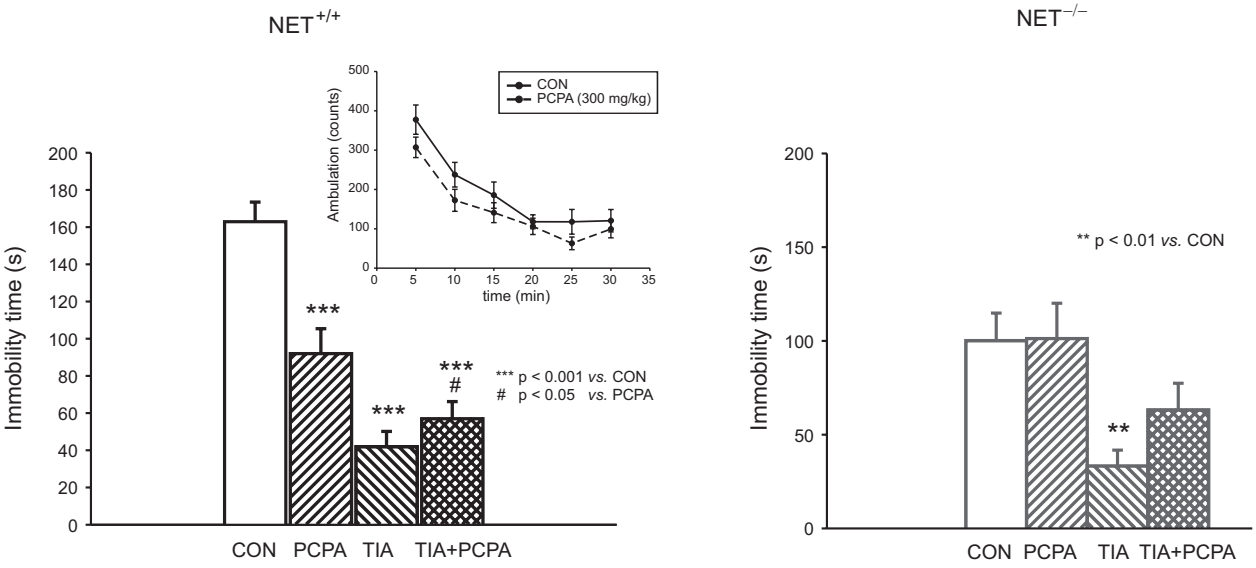

Fig. 4. Effect of tianeptine (TIA, 20 mg/kg) and PCPA (300 mg/kg) on immobility time in NET$^{+/+}$ and NET$^{-/-}$ mice subjected to forced swim test (FST). Results are the mean immobility time ± SEM. The groups consisted of 10 animals each. Statistical analysis was performed by one-way ANOVA followed by Bonferroni *post-hoc* analysis. INSET: Effect of PCPA (300 mg/kg) on locomotor activity in NET$^{+/+}$ mice. Results are the mean ambulation counts ± SEM. The groups consisted of 10 animals each. Statistical analysis was performed by Student's *t*-test

sponses of mice subjected to TST and FST. The time-dependent response of plasma corticosterone following these two behavioral challenges remain to be elucidated, particularly in the context of strain-specific differences [7].

In the present study, the response of NET$^{-/-}$ mice (as far as the plasma level of corticosterone) was less pronounced than that of control animals, confirming their depression-resistant phenotype (shown previously [4, 13]). These mice have also been reported to be less vulnerable to seizures [8]. Therefore, the effect of tianeptine in the FST could be explained by the influence of that drug on the level of corticosterone (i.e., the stronger normalizing effect of tianeptine on the elevated level of corticosterone and more pronounced effect in the FST observed in NET$^{-/-}$ mice *vs.* control). By analogy – the lack of an effect following tianeptine treatment in control mice subjected to TST might be explained by the much weaker response of these animals to TST, as far as the level of corticosterone. Nonetheless, tianeptine is very active in the NET$^{-/-}$ mice subjected to TST, despite unchanged plasma levels of corticosterone in response to that test. Therefore, the "antidepressant effect of tianeptine" cannot be unequivocally credited to a normalizing influence on the hyperactive HPA axis. More biochemical studies are necessary to elucidate the mechanism of action of tianeptine, which may prove to be an antidepressant drug of non-conventional pharmacological profile [9]. Such knowledge would further our understanding of the neurobiological mechanisms involved in depression.

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