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ScienceDirect
Pharmacology, Biochemistry and Behavior 85 (2006) 813–819
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
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Effect of acute and repeated treatment with mirtazapine on the immunity of noradrenaline transporter knockout C57BL/6J mice

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Received 9 November 2005; received in revised form 12 October 2006; accepted 22 November 2006
Available online 29 December 2006

Abstract

Pathological immunoactivation is thought to play an important role in the etiology of depression; however, the effect of novel antidepressant drugs on immunity has been poorly recognized. Mirtazapine, an antidepressant drug, enhances noradrenergic and serotonergic neurotransmissions, which are crucially involved in the regulation of immune system activity. In the present study we examined the effect of acute and seven-day repeated administration of mirtazapine (20 mg/kg, i.p.) on immunoreactivity in noradrenaline transporter knockout (NET-KO) and wild-type male C57BL/6J mice subjected to the forced swimming test (FST). Mirtazapine decreased immobility time in the FST after acute, but not seven-day repeated, administration to C57BL/6J mice. Lack of the antidepressant effect of mirtazapine was observed, after acute and repeated administration to NET-KO mice, although those mice showed a significantly shorter immobility time in the FST than did wild-type animals. Seven-day repeated mirtazapine administration to wild-type mice suppressed the proliferative activity of splenocytes and their ability to produce pro-inflammatory cytokines, whereas production of IL-4 was stimulated. Acute mirtazapine administration did not change immune parameters in C57BL/6J mice. In NET-KO mice, acute and seven-day repeated mirtazapine administration reduced the proliferative activity of splenocytes and their ability to produce pro-inflammatory cytokines. This study indicates that, in comparison with wild-type C57BL/6J mice, NET-KO mice show enhanced mobility, which is not further potentiated by mirtazapine treatment. Furthermore, the NET-KO mice display higher susceptibility to the immunosuppressive effects of mirtazapine than do the wild-type animals. The present paper postulates an essential role of noradrenergic system in the immunological and behavioral effects of mirtazapine.

Keywords: Mirtazapine; Cytokines; Proliferation of lymphocytes; Forced swimming test; Noradrenaline transporter knockout mice

1. Introduction

The immunological theory of depression suggests that pathological immunoactivation e.g. an increased production of pro-inflammatory cytokines, plays an important role in the etiology of depression (Smith, 1991; Maes et al., 1995). A number of effects of pro-inflammatory cytokines in animals and patients subjected to cytokine immunotherapy resemble those observed in depression, namely reduced food intake (anorexia) and locomotor activity (motor retardation) and the ability to feel pleasure (anhedonia). A brain neuroimaging study with patients treated with cytokines showed changes similar to those observed in depressed patients (Meyers et al., 1994). Moreover, pro-inflammatory cytokines activate the hypothalamic–pituitary–adrenal axis (HPA), and depression is usually associated with the hyperactivation of the HPA axis.

If the increased level of pro-inflammatory cytokines is involved in the etiology of depression, it may be expected that antidepressants should have a negative immunoregulatory

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doi:10.1016/j.pbb.2006.11.017

effect. Indeed, it has been shown that antidepressants dose-dependently inhibit the production of pro-inflammatory cytokines by lymphocytes and monocytes (Xia et al., 1996; Maes et al., 1999; Kubera et al., 2001). Pharmacological studies with noradrenaline transporter (NET) inhibitors such as desipramine (Kubera et al., 1998; Selmeczy et al., 2003) have indicated the involvement of the noradrenergic system underlying the effects of antidepressants on immune activity.

Mirtazapine has been described as a safe and effective new generation antidepressant drug which enhances noradrenergic neurotransmission via an antagonistic action at α_2-adrenergic autoreceptors and heteroreceptors. It is well documented that the sympathetic system may modulate – via noradrenaline (NE) – cell-mediated immunity. Sympathetic nerve terminals that innervate lymphoid organs are equipped with release-regulating presynaptic α_2 adrenoreceptors, which mediate the negative feedback of NE release (Elenkov et al., 2000). Some immune cells express adrenoreceptors, NE may exert its effect on the immune system both directly – mainly through β_2 adrenoreceptors – and indirectly — via modulation of its own release (Szelenyi and Selmeczy, 2002). By its antagonistic action on presynaptic α_2 adrenoreceptors, mirtazapine may increase noradrenaline level in the lymphocyte environment and affect cell-mediated immunity.

Mirtazapine has many salutary features: for example, it reduces hypercortisolemia, increases at stage IV sleep, and exerts potent anti-nausea effects, which stimulate appetite in cancer patients (Anttila and Leinonen, 2001; Kast, 2001). On the other hand, the effect of mirtazapine on the immune system is scarcely recognized. To the best of our knowledge, only the effect of chronic mirtazapine administration on the level of the tumor necrosis factor (TNF)-α and its receptors in depressed patients has been studied so far. Mirtazapine increased the circulating level of TNF-α and its receptors (Kraus et al., 2002). On the basis of the latter observation it was suggested that the use of mirtazapine could be dangerous for depressed patients suffering from an illness in which the increased level of TNF-α may have a pathogenic effect, e.g. rheumatoid arthritis or idiopathic dilated cardiomyopathy. Furthermore, it was suggested that mirtazapine ought to be regarded as a drug of first choice during cancer immunotherapy or during a mycobacterial infection (Kast, 2003). Thus description of the immunomodulatory effect of the antidepressant on several other immunological parameters would be helpful to give a better rationale for its choice in sickness disorders coexisting with depression. This study describes the effect of single or seven-day repeated mirtazapine treatment on some parameters of cell-mediated immunity such as the ability of splenocytes to proliferate in response to mitogens and to produce pro- and anti-inflammatory cytokines in C57BL/6J and noradrenaline transporter-deficient (NET-KO) mice, as it has been suggested that a noradrenaline transporter plays a crucial role in the regulation of the cell-mediated immune response (Selmeczy et al., 2003). Concurrently, the effects of mirtazapine treatment in these mice have been evaluated in the forced swim test (FST), which is an established procedure used to assess the effects of antidepressants on depressed-like behaviors relevant to clinical depression (Porsolt et al., 1977).

2. Materials and methods

2.1. Mice and drug administration

Wild-type C57BL/6J mice and their genetically modified NET−/− counterparts (NET-KO) were obtained from Prof. Heinz Bonisch's laboratory at the Institute of Pharmacology and Toxicology, the University of Bonn, Germany and maintained in the Animal Department of the Institute of Pharmacology, Polish Academy of Science in Kraków, Poland (Gilsbach et al., 2004a,b). The animals were kept under standard animal house conditions (at a room temperature of 23 °C, on a 12/12 h light/dark cycle, the light on at 08:00), with food and water *ad libitum*. Two-month old C57BL/6J and NET-KO male mice randomly divided into 3 groups (control, "acute mirtazapine", "7-day repeated mirtazapine"), and were acclimatized for at least 1 week before the experiment. All the treatments conformed to the NIH Animal Care Guide and were approved by the Local Bioethics Commission. Mirtazapine (Organon, The Netherlands) was suspended in a 1% aqueous solution of Tween 80 and was administered intraperitoneally (i.p.) at 8 a.m. once (acute treatment) or repeatedly (daily, for 7 days) in a dose of 20 mg/kg. Control animals received the vehicle through the whole experimental period, also the animals receiving single injections of mirtazapine were treated repeatedly (daily, for 6 days) with the vehicle, whereas mirtazapine was administered on day 7.

2.2. Forced swimming test (FST)

The mice were dropped into glass beakers (2 l), filled with water (21–23 °C) up to a height of 6 cm, 1 h after a single dose or the last injection (repeated treatment) of the compound studied. The immobility time was assessed during the last 4 min of a 6 min test according to the method described by Porsolt et al. (1977). Water in the beakers was changed for each mouse.

2.3. Proliferation assay and cytokine detection and quantification

The proliferative response of spleen cells, cytokine detection and quantification were described in our earlier paper (Kubera et al., 2000). The mice were decapitated 2 h after the last vehicle/drug injection and 1 h after the FST. Their spleens and thymuses were aseptically dissected and weighed. The spleens were gently crushed in a glass homogenizer. Cells were suspended in RPMI-1640 medium and were centrifuged at 500 g for 5 min. The cell pellets were re-suspended in the same medium supplemented with antibiotics and 10% fetal calf serum**.** Spleen cells (4×10^6 cells/ml) were stimulated by optimal concentrations of concanavalin A (Con A; 1.25 μg/ml) and lipopolysaccharide (LPS, 5 μg/ml) and were incubated in 96-well plates at final volume of 0.2 ml for 72 h. Cell proliferation was determined by adding 0.5 μCi of [^{3}H]-thymidine per well (ICN, USA; Spa 6.7 Ci/mmol) at 16 h before the end of the incubation. The production of IL-4, IL-6 and IFN-γ was examined after 48 h of Con A (1.25 μg/ml) stimulation.

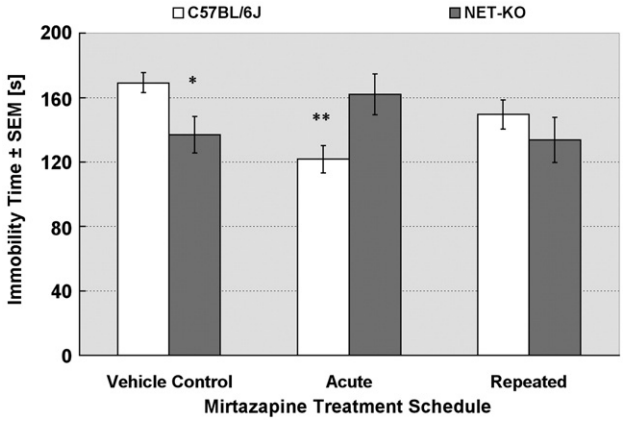

Fig. 1. Effect of acute or seven-day repeated mirtazapine administration on the immobility time of C57BL/6J and NET-KO mice in the forced swimming test (FST). The last doses of vehicle and mirtazapine (20 mg/kg) were administered 1 h before the FST. The animals were observed for 4 min. The results are the mean of immobility time ± SEM [s]; $n=5$, *$p<0.05$ and **$p<0.01$ *vs* C57BL/6J mice treated with vehicle.

Cytokines were measured by specific ELISA, using pairs of anti-cytokine Mo-Abs (PharmMingen).

2.4. Statistical analysis

Behavioral studies were carried out on six groups of animals (10 mice per group). For immunological studies five animals were randomly chosen from each group. Behavioral and immunological data were evaluated by two-way analysis of variance (ANOVA) (2 strains × 3 treatment conditions) followed, when appropriate, by *post hoc* LSD Fisher's test. p values lower than 0.05 were considered statistically significant.

3. Results

3.1. Behavioral studies

Two-way ANOVA showed that neither strain ($F(1,54)=0.09$; $p=0.767$) nor drug treatment ($F(2,54)=0.70$; $p=0.469$) affected immobility time significantly. However, a significant interaction between these factors was noted ($F(2,54)=6.45$; $p<0.003$). *Post hoc* analysis (with LSD Fisher's test) revealed that vehicle-treated NET-KO mice showed significantly lower immobility time than vehicle-treated C57BL/6J mice (Fig. 1; $p<0.05$). Also, acute, but not repeated administration of mirtazapine reduced immobility time in C57BL/6J mice ($p<0.01$). Neither acute nor repeated mirtazapine treatment affected immobility time of NET-KO mice significantly.

3.2. Immunological studies

Two-way ANOVA showed that neither strain ($F[1,23]=0.0002$; $p=0.99$) nor drug treatment ($F[2,23]=1.30$; $p=0.29$) affected relative thymus weight significantly. However, a significant interaction between these factors was noted ($F[2,23]=3.44$; $p<0.049$). *Post hoc* analysis revealed that mirtazapine increased the relative thymus weight of C57BL/6J mice significantly only following repeated treatment (Table 1; $p<0.02$). However, neither acute nor repeated treatment affected relative thymus weight of NET-KO mice. Relative spleen weight was not affected by mirtazapine in either C57BL/6J or NET-KO mice (Table 1).

Table 1
Effect of mirtazapine administration on the weight of thymuses and spleens obtained from wild-type or noradrenaline transporter knockout C57BL/6J mice (NET-KO)

Strain of mice	Mirtazapine treatment schedule	Relative thymus weight mean ± SEM	Relative spleen weight mean ± SEM
C57BL/6J	Vehicle control	1.25 ± 0.07	4.25 ± 0.21
	Acute	1.63 ± 0.12	4.07 ± 0.21
	Repeated	**1.85 ± 0.20***	5.33 ± 0.96
NET-KO	Vehicle control	1.60 ± 0.18	4.88 ± 0.33
	Acute	1.77 ± 0.24	5.29 ± 1.42
	Repeated	1.35 ± 0.15	4.74 ± 0.46

Data expressed as the mean ± SEM ($n=5$). *$p<0.02$ *vs* vehicle-treated C57BL/6J mice. Relative thymus or spleen weight was calculated as ratio of weight of thymus or spleen (mg) to weight of body (g).

Two-way ANOVA revealed that NET-KO mice showed significantly lower proliferative activity of splenocytes in response to Con A relative to C57BL/6J mice (mean ^{3}H-thymidine incorporation 57287 *vs* 72912 dpm, respectively; F strain $[1,24]=11.51$; $p<0.002$). Mirtazapine treatment significantly suppresses proliferative activity of splenocytes in response to Con A ($F[2,24]=40.51$; $p<0.001$). *Post hoc* main effect analysis indicated that only repeated administration of the drug exerted suppressive effect (Fig. 2, inset; $p<0.001$). Also, a significant strain by treatment interaction ($F[2,24]=20.58$; $p<0.001$) was observed. *Post hoc* analysis showed that both acute and repeated mirtazapine treatment reduced Con A-induced splenocyte proliferative activity in NET-KO mice (Fig. 2;

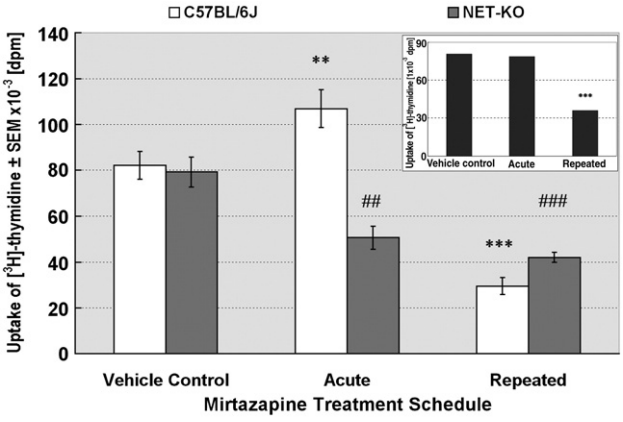

Fig. 2. Effect of acute or seven-day repeated mirtazapine administration on the proliferation of Con A-stimulated splenocytes obtained from C57BL/6J and NET-KO mice subjected to the forced swimming test (FST). The splenocytes were collected 2 h after the last dose of vehicle and mirtazapine (20 mg/kg) and 1 h after the FST test. The data are presented as the mean ± SEM [dpm]; $n=5$. **$p<0.01$ and ***$p<0.001$, *vs* C57BL/6J mice treated with vehicle; $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$, respectively, *vs* NET-KO mice treated with vehicle as evaluated by LSD Fisher's test. The inset figure illustrates main mirtazapine treatment effect on Con A-induced proliferative activity of splenocytes. ***$p<0.001$ against vehicle control.

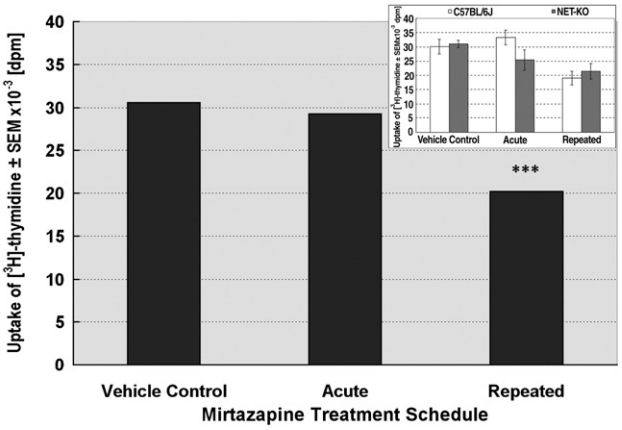

Fig. 3. Effect of acute or seven-day repeated mirtazapine administration on the proliferation of LPS-stimulated splenocytes obtained from C57BL/6J and NET-KO mice subjected to the forced swimming test (FST). The splenocytes were collected 2 h after the last dose of vehicle and mirtazapine (20 mg/kg) and 1 h after the FST test. The data are presented as the mean±SEM [dpm]; $n=5$. The significance of the main treatment effect was evaluated by LSD Fisher's test where ***$p<0.001$ compared against vehicle control. The effects of mirtazapine treatment on the individual treatment means are shown on the inset figure for illustration purposes only.

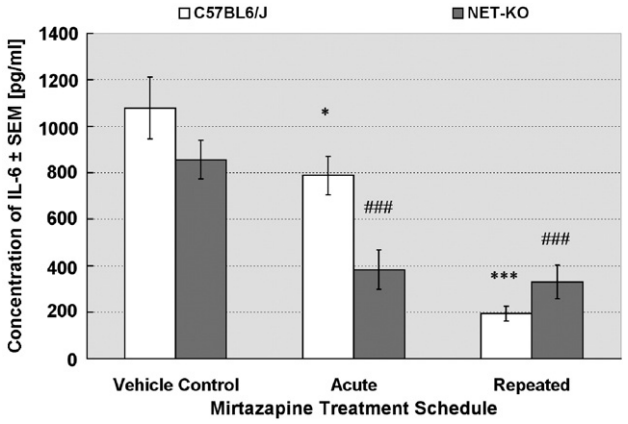

Fig. 5. Effect of acute or seven-day repeated mirtazapine administration on the IL-6 production by Con A-stimulated splenocytes obtained from C57BL/6J and NET-KO mice subjected to the forced swimming test (FST). The splenocytes were collected 2 h after the last dose of vehicle and mirtazapine (20 mg/kg) and 1 h after the FST test. The data are presented as the mean±SEM [pg/ml]; $n=5$. *$p<0.05$ and ***$p<0.001$ *vs* C57BL/6J mice treated with vehicle; ###$p<0.001$ *vs* NET-KO mice treated with vehicle.

$p<0.01$ and $p<0.001$, respectively). In the case of C57BL/6J mice, Con A-splenocyte proliferative activity was reduced only by repeated mirtazapine treatment ($p<0.001$). Single administration of mirtazapine to C57BL/6J mice exerted even stimulatory effect ($p<0.01$). There was no difference between C57BL/6J and NET-KO vehicle-treated mice.

Mirtazapine treatment significantly suppress proliferative activity of splenocytes in response to LPS ($F[2,24]=9.34$; $p<0.001$). *Post hoc* main effects analysis indicated that seven-day repeated, but not acute, mirtazapine administration to both C57BL/6J and NET-KO mice reduced the proliferative activity of splenocytes in response to LPS (Fig. 3; $p<0.001$). There were no significant strain ($F[1,24]=0.52$; $p=0.48$) or strain by treatment interaction ($F[1,24]=2.28$; $p=0.12$). The effects of mirtazapine treatment on the individual treatment means are shown on the inset figure for illustration purposes only (Fig. 3).

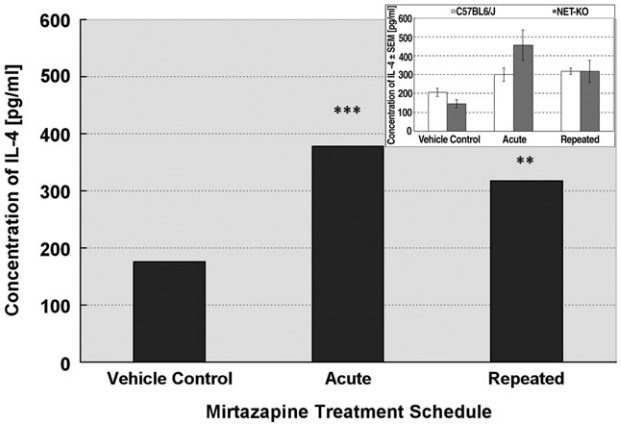

Fig. 4. Effect of acute or seven-day repeated mirtazapine administration on the IL-4 production by Con A-stimulated splenocytes obtained from C57BL/6J and NET-KO mice subjected to the forced swimming test (FST).The splenocytes were collected 2 h after the last dose of vehicle and mirtazapine (20 mg/kg) and 1 h after the FST test. The data are presented as the mean±SEM [pg/ml]; $n=5$. The significance of the main treatment effect was evaluated by LSD Fisher's test where **$p<0.01$ and ***$p<0.001$ compared against vehicle control. The effects of mirtazapine treatment on the individual treatment means are shown on the inset figure for illustration purposes only.

Two-way ANOVA showed that mirtazapine treatment significantly enhanced the production of IL-4 by Con A-stimulated splenocytes ($F[2,24]=10.53$; $p<0.001$). *Post hoc* main effects analysis indicated that single as well as repeated administration of mirtazapine stimulated synthesis of IL-4 (at the level of $p<0.001$ and $p<0.01$, respectively) in both C57BL/6J and NET-KO mice (Fig. 4). There were no significant strain ($F[1,24]=0.77$; $p<0.39$) or strain by treatment interaction ($F[1,24]=3.07$; $p<0.07$). The effects of mirtazapine treatment on the individual treatment means are shown on the inset figure for illustration purposes only (Fig. 4).

Mirtazapine significantly reduced IL-6 production by Con A-stimulated splenocytes (control:acute:repeated::967:586:264 mean IL-6 production in pg/ml; F treatment $[2,24]=33.01$, $p<0,001$). *Post hoc* main effect analysis showed that single as well as repeated administration of mirtazapine suppressed synthesis of IL-6 (at the level of $p<0.001$ and $p<0.01$, respectively). There was also a significant strain effect on IL-6 production (C57BL/6J:NET-KO::687:524 mean IL-6 production in pg/ml, F treatment $[1,24]=5.33$; $p=0.03$). A significant strain by treatment interaction ($F[2,24]=5.02$; $p=0.02$) indicated that while both acute and repeated mirtazapine treatment reduced IL-6 production in NET-KO mice similarly (mean IL-6 concentration 384 and 332 pg/ml, respectively; $p<0.001$), in the case of C57BL/6J mice repeated treatment had considerably strong suppressive effect than that exerted by acute administration (Fig. 5; mean IL-6 concentration 788 and 195 pg/ml, respectively; $p<0.05$ and $p<0.001$, respectively).

Mirtazapine significantly inhibited IFN-γ production by Con A-stimulated splenocytes (control:acute:repeated::449:371:307 mean IFN-γ production in pg/ml; F treatment $[2,24]=30.36$;

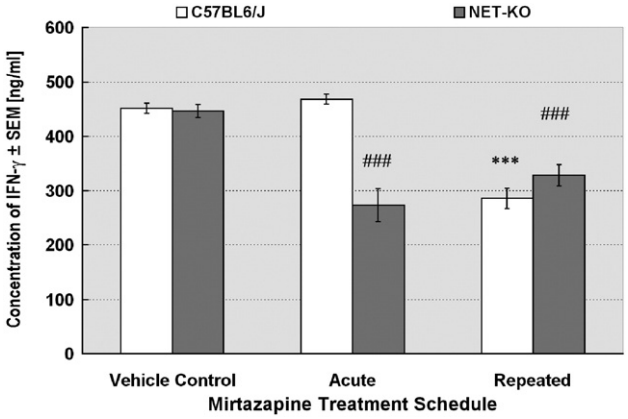

Fig. 6. Effect of acute or seven-day mirtazapine administration on the IFN-γ production by Con A-stimulated splenocytes obtained from C57BL/6J and NET-KO mice subjected to the forced swimming test (FST). The splenocytes were collected 2 h after the last dose of vehicle and mirtazapine (20 mg/kg) and 1 h after the FST test. The data are presented as the mean±SEM [ng/ml]; $n=5$. ***$p<0.001$ *vs* C57BL/6J mice treated with vehicle, $^{###}p<0.001$ *vs* NET-KO mice treated with vehicle.

$p<0.001$). *Post hoc* main effect analysis revealed that both acute and repeated treatment exerted suppressive effect in C57BL/6J and NET-KO mice ($p<0.001$). There was also a significant strain effect on IFN-γ production (C57BL/6J:NET-KO::402:349 mean IL-6 production in pg/ml, $F[1,24]=12.39$; $p<0.002$). A significant strain by treatment interaction ($F[2,24]=23.69$; $p<0.001$) indicated that while both acute and repeated mirtazapine treatment reduced IFN-γ production in NET-KO mice (Fig. 6; $p<0.001$), only repeated administration was capable to reduce IFN-γ production in C57BL/6J mice ($p<0.001$).

4. Discussion

Decreases in noradrenergic transmission are implicated in the neurochemical mechanism of depression. We observed that struggling responses in the FST model of depression-like behaviors were more robust in NET-KO mice than in C57BL/6J mice. Furthermore, mutant mice showed behavioral changes which resembled those induced by antidepressants in C57BL/6J mice. The behavioral data are consistent with the proposed role of NE in depression, and confirm the observations made by Xu et al. (2000) in the FST using SvJ NET-KO mice. Moreover, in the present study, the NET-KO mice were less active in the open field suggesting that their enhanced response in the FST was not due to a generalized increase in activity (data not shown).

Deletion of the NET was associated with decreased immobility in the FST. However, neither acute nor seven-day repeated administration of mirtazapine produced any additional changes in NET-KO behavior. Similar effects were reported by Xu et al. (2000) in NET-KO mice subjected to the tail suspension test; an analogue of the FST (Cryan et al., 2005). In this case NET-KO mice also showed enhanced struggling under vehicle conditions without any further changes as a function of desipramine treatment. These observations suggest that the intensification of noradrenergic transmission as a result of genetical disruption of the NET gene efficiently protects animals against depressive-like changes and this effect is not increased by administration of antidepressants.

The main finding of this paper concerns the immunosuppressive effect of mirtazapine on the cell-mediated immunity of mice subjected to forced swimming stress. In wild-type C57BL/6J mice, an inhibitory effect on the immune system activity was observed after seven daily injections of mirtazapine, whereas in mice without the NE transporter such an effect was observed after both single and repeated mirtazapine injection, which suggests higher susceptibility of mutant mice to mirtazapine-induced changes in the immune system activity.

The immunosuppressive activity of mirtazapine is probably not connected with a direct effect of this drug on the α_2-adrenergic receptor, since the presence of this receptor on immune cells is still a matter of controversy. However, some functional studies point to the implication of α_2 receptors in the modulation of some immune parameters, and suggest that under certain pathological conditions, α_2 receptors are expressed on immune cells (Heijnen et al., 1996). Moreover these functional studies show that inhibition of α_2-adrenergic receptor should result in immunoactivation rather than immunosupression. On the other hand, the activation of α_2-adrenergic receptors leads to suppression of peripheral blood T-cell reactivity and reduces the number of several subclasses of lymphocytes (Stevenson et al., 2001).

Mirtazapine exhibits potential ability to increase the level of noradrenaline in spleen environment. Noradrenaline inhibits its own release via α_2 adrenoreceptors (autoreceptors) present on noradrenergic terminals in the spleen whereas mirtazapine is expected to induce an opposite effect by the blockade of α_2 adrenoreceptors. It has been well documented that noradrenaline modulates immune functions mainly by β-adrenergic receptors present on all lymphoid cells, except for T helper 2 cells. Several lines of evidence suggest that catecholamines – via β-adrenergic receptors – increase of cAMP amount in T cells and inhibit their proliferation induced by mitogens (Elenkov et al., 2000). The proliferative response of $CD8^+$ T cells is inhibited to a higher extent than in the case of $CD4^+$ T cells, presumably because $CD8^+$ T cells have a greater number of β-adrenergic receptors (Elenkov et al., 2000; Bartik et al., 1993).

The pharmacological profile of mirtazapine is characterized by not only α_2-adrenergic, but also 5-HT_2-, 5-HT_3- and histamine H_1 receptor antagonistic activities. On the other hand, the function of immune cells is influenced by not only adrenergic receptors, but also different types of receptors which commonly do not operate independently of each other. Macrophages mediate their accessory function in T-cell activation via 5-HT_2 receptors by increasing the expression of IL-2R on T cells and their proliferation in response to Con A (Kut et al., 1992; Young et al., 1993; Young and Matthews, 1995). On the other hand, it has been shown that the proliferation of mouse T cells after Con A stimulation can be reduced by 5-HT_2 receptor antagonists (Young et al., 1993). Also H_1 receptor antagonists are capable of inhibiting T-cell proliferation (Roberts et al., 1994; Radvany et al., 2000). Hence

it is concluded that the inhibition of the activity of 5-HT_2 and/or H_1 receptors on lymphocytes and/or macrophages by mirtazapine maybe responsible for attenuation of the proliferative activity of spleen cells.

Despite noradrenaline-dependent differences in the behavior of NET-KO and wild-type mice and the important role of noradrenaline in the modulation of the immune system, in the present study we did not find any alterations in the majority of parameters of cell-mediated immunity between NET-KO and wild mice. It should be noted, however, that such compensatory mechanisms as perturbations in other monoamine-uptake systems e.g. in the dopamine-uptake system are frequently observed in genetically modified NET-KO animals; it is also possible that these mechanisms permit mutant animals to maintain parameters of immunity on the level observed in wild-type mice. It has been shown that the absence of the NET gene is accompanied with supersensitivity of dopamine D_2/D_3 receptors and that the decreased in cAMP level due to the activation of dopamine D_2/D_3 receptors (Xu et al., 2000) might compensate for the increase in cAMP level as a result of the increase noradrenaline action on β-adrenergic receptors on lymphocytes in NET-KO mice.

In the present study mirtazapine administration inhibited the production IFN-γ, a cytokine typical of Th1 cells, and increased the production of IL-4, a cytokine characteristic of Th2 cells. An increasing body of evidence suggests that catecholamines inhibit selectively Th1 cell functions and favor Th2 cell responses rather than cause generalized immunosuppression, as was previously believed. The inhibition of IFN-γ production observed in our study probably results directly from the inhibitory effect of NE on Th1 cell function and indirectly from the inhibition of macrophage activity and their ability to produce main inducers of Th1 cell responses, e.g. IL-12.

The Th1 and Th2 cell responses are mutually inhibitory. Thus, although noradrenaline does not modulate directly the Th2 cell function due to the lack of β-adrenergic receptors on these cells, it may indirectly stimulate IL-4 production by inhibiting the Th1 response. The stimulatory effect of mirtazapine administration on IL-4 production described in our study could be at least partly due to the increase in NE level in the splenocyte environment. Also application of other-than-mirtazapine α_2-adrenergic antagonists, such as CH-38083, prazosine or WB-4101, results in the inhibition and activation of Th1 and Th2 cells, respectively (Elenkov et al., 1995; Szelenyi et al., 2000a,b). In mice, pretreatment with salbutamol, a β_2-adrenergic agonist, induces an increase in the *ex vivo* release of IL-4 and IL-10 from concanavalin-A-activated splenocytes (Coqueret et al., 1994). In both murine and human systems, β_2-adrenergic receptor agonists and exogenous catecholamines inhibit the IFN-γ production by Th1 cells and the production of IL-12 by macrophages (Borger et al., 1998). On the other hand, application of propranolol, a β-adrenergic antagonist results in substantial increases in the mitogen-induced cytokine secretion by Th1 cells (Elenkov et al., 1995; Hasko et al., 1995).

IL-6 is produced by several cells and its production by Th2 cells needs co-stimulatory activity of macrophages. As has been mentioned above, macrophages are particularly sensitive to the suppressive effect of noradrenaline. Therefore in the present study the inhibition of IL-6 production in mirtazapine-treated wild-type and NET-KO mice can be explained by the mirtazapine-induced increase in NE level in the spleen.

In summary, this study indicates that NET-KO mice show enhanced mobility in the FST, which is not further potentiated by mirtazapine treatment. Furthermore, these mice show higher susceptibility to the immunosuppressive effect of mirtazapine than do wild-type animals. The stronger modulatory effect of mirtazapine on the parameters of cell-mediated immunity in NET-KO mice compared to wild-type ones may be due to a higher concentration of noradrenaline in the splenocyte environment, caused by the summary effect of the lack of NE transporter and blockade of α_2 receptors. Our study confirms the essential role of the noradrenergic system in the immunological and behavioral effects of mirtazapine. Moreover, a strong inhibitory effect after repeated mirtazapine administration testifies to the necessity of monitoring cell-mediated immunity, especially in persons subjected to other agents stimulating the sympathetic system, e.g. stress, or to other inhibitors of noradrenaline reuptake.

Acknowledgement

This study was supported by the grant 6P05A076 20 from the State Committee for Scientific Research (KBN), Warszawa, Poland.

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