Chronic antidepressant treatment exerts sexually dimorphic immunomodulatory effects in an experimental model of major depression: do females lack an advantage?

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Abstract

Major depression is a stress-related disorder that affects about 20% of the population, with women outnumbering men by 2:1 (Berton & Nestler, 2006). Patients often show alterations in responses of both the innate and the cell-mediated arms of immunity that are associated with infectious-disease susceptibility (Zorrilla et al., 2001). However, research focusing on stress/antidepressant-related immunomodulation often neglects sex differences (Darnall & Suarez, 2009), although a well-established sexual dimorphism also characterizes the immune system (De Leon-Nava et al., 2009). Chronic mild stress (CMS) is a widely accepted animal model of MD, previously reported to induce behavioural, neurobiological and immunological alterations in male rodents (Willner, 2005). Chronic mild stress (CMS) application in rats, exert sexually dimorphic effects on cellular immunoreactivity (natural killer and lymphokine-activated killer cell cytotoxicity and interleukin-2-induced T-cell proliferation), with females presenting a relatively immunosuppressed phenotype compared to males. Moreover, following chronic antidepressant treatment, thymic monoamines presented sex-related alterations, as well as intriguing associations with peripheral T-cell responses. This study highlights the sex-related effects of chronic clomipramine treatment and CMS application on the cellular arm of immunity, and represents a preliminary exposé of a thymus-dependent route pertaining to the interactions between antidepressants and the immune system.

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Introduction

Major depression (MD) affects about 20% of the population, with women outnumbering men by 2:1 (Berton & Nestler, 2006). Patients often show alterations in responses of both the innate and the cell-mediated arms of immunity that are associated with infectious-disease susceptibility (Zorrilla et al., 2001). However, research focusing on stress/antidepressant-related immunomodulation often neglects sex differences (Darnall & Suarez, 2009), although a well-established sexual dimorphism also characterizes the immune system (De Leon-Nava et al., 2009). Chronic mild stress (CMS) is a widely accepted animal model of MD, previously reported to induce behavioural, neurobiological and immunological alterations in male rodents (Willner, 2005). Most importantly, ongoing research in our laboratory indicates that the phenotypes employed by rats subjected to models of depression are sexually dimorphic (Dalla et al., 2008; Drossopoulou et al., 2004; Kamper et al., 2009; Pitychoutis et al., 2009).

The interplay between the brain and the immune system is primarily depicted in the fact that the thymus is innervated by post-ganglionic sympathetic nerve fibres that release catecholamines (CAs) in the vicinity of both thymocytes and non-lymphoid thymic cells (Qiu et al., 1996). MD and CMS have both been associated with alterations regarding central and peripheral monoaminergic systems, as well as T-cell-dependent immunity (Bekris et al., 2005; Dalla et al., 2008; Kubera et al., 1998; Willner, 2005; Zorrilla et al., 2001). Most importantly, monoamines are known regulators of T-cell differentiation/maturation in the thymus (Leposavic et al., 2008). However, the role of the thymus, the central T-cell pool, has been overlooked in studies screening for immunomodulatory T-cell responses in several stress models that instead have only focused on peripheral immune responses (Frick et al., 2009; Kubera et al., 1998). The effects of
antidepressants on immune cells have been postulated to be mediated by both direct and indirect/compensatory routes that are not completely understood but comprise a matter of intensive research (Fazzino et al. 2009; Frick et al. 2009).

Given the immune sexual dimorphism and the established ‘gender’ component of MD, we hypothesized that cellular responses of thymus-dependent (T-cells) and/or thymus-independent [natural killer (NK) cells] immune components may be affected in a sex-related manner, following chronic antidepressant treatment in the CMS model. Since the thymus is a stress-sensitive immune organ actively participating in cell-mediated neuroimmune cross-talks, we sought to generate its monoamine profile and investigate whether alterations of the thymic monoamine milieu would be associated with peripheral T-cell immunomodulatory responses in view of chronic antidepressant treatment.

Method

Animals and CMS procedure

Male \((n = 14)\) and female \((n = 14)\) Sprague–Dawley rats obtained from the Hellenic Pasteur Institute (Athens, Greece) were subjected to CMS, while an equal number of rats \((n = 14\) per sex) served as controls. CMS lasted for 7 wk and consisted of 2–5 different stressors per day, as previously described (Bekris et al. 2005). In brief, the stressors were: food and/or water deprivation, cage tilt, continuous lighting, soiled cage, grouped housing in one cage, stroboscopic illumination, intermittent white noise, odour, foreign object in cage, low room temperature, restricted access to food, empty water bottles. On week 4 of the stress regime, each control and CMS group was further divided into two groups, thus resulting in four subgroups \((n = 7\) per subgroup): two control groups that received saline injections as vehicle (Control-Veh) or clomipramine (Control-Drug), respectively, a CMS group that received Veh injections (CMS-Veh), and a CMS group that received clomipramine (CMS-Drug). Clomipramine (amp Anafranil 25 mg/ml, Novartis Hellas, Greece) was obtained from a local drug store. Animal experiments were carried out in accordance with the National Institute of Health’s Guide for the Care and Use of Laboratory Animals (NIH Publications No.80–23), revised 1996.

Determination of thymic monoamines

Noradrenaline (NA), dopamine (DA) and serotonin (5-hydroxytryptamine; 5-HT) were determined by high-performance liquid chromatography with electrochemical detection (HPLC-ED; Pitychoutis et al. 2009). Following sacrifice, both thymic lobes were carefully removed, placed on ice, immediately weighed and processed as previously described (Pilipovic et al. 2008).

\[^{3}H\]thymidine incorporation assay

Splenocytes were isolated from individually homogenized spleens, and incubated in plain medium (RPMI-1640, 10% foetal bovine serum; 18 h, basal proliferation) or in the presence of 50 IU/ml recombinant mouse interleukin-2 (IL-2; R&D Systems GmbH, Germany; 72 h), which enhances T-cell-mediated responses. Proliferation indices were calculated according to the formula: c.p.m. of IL-2-exposed culture/c.p.m. of basal culture. All measurements were made in triplicate.

NK and lymphokine-activated killer (LAK) cell cytotoxicity

The cytotoxicity of splenocytes was tested against the YAC-1 lymphoma (NK-sensitive) and the P815 mastocytoma (LAK-sensitive) cell targets. LAK effectors, also comprising activated T-cells, were generated upon incubation of splenocytes with IL-2 (250 IU/ml) for 5 d. Target cells (YAC-1 and P815) were \(^{51}\)Cr-labelled according to Baxevanis et al. (2000) and co-cultured with effectors at a ratio of 100:1 for 18 h at 37 °C. Isotope release was determined and percentage of specific cytotoxicity was calculated as previously described (Baxevanis et al. 2000). All measurements were made in triplicate.

Statistical analysis

Three-way analysis of variance (ANOVA) with stress, sex and drug as independent factors was used for screening statistically significant differences regarding the immune parameters assayed. In view of significant
interactions, subsequent one-way ANOVAs were implemented to elucidate specific differences between groups. Correlation analysis was undertaken by estimating Pearson’s correlation coefficients for multiple comparisons.

Results

Relative thymus weight

Under basal conditions, female rats were characterized by higher relative thymus weight compared to their male counterparts. Chronic clomipramine treatment induced a female-specific decrease of the relative thymus weight both in control and CMS-treated rats, while CMS application had no effect on either sex (Fig. 1a).

Monoamine profile of the thymus

Female rats were characterized by lower thymic NA concentrations compared to male rats but NA levels were not affected by either drug administration or CMS application. Conversely, chronic clomipramine treatment induced a male-specific enhancement of NA thymic concentrations both in CMS and control rats (Fig. 2a).

Similarly, female rats were characterized by lower thymic DA concentrations compared to male rats at baseline. In male rats, stress or drug administration did not alter thymic DA levels in a statistically significant manner. CMS application increased thymic DA levels in CMS-Veh female rats; this increase was partially reversed by chronic clomipramine treatment (Fig. 2b).
Chronic clomipramine treatment induced an increase in thymic 5-HT concentrations in both sexes. CMS application did not cause any relevant alterations in 5-HT levels of the thymus in either sex, but reduced the boosting of 5-HT levels that was evidenced in control, clomipramine-treated rats (Fig. 2c).

**IL-2-induced T-cell proliferation**

In the control group, chronic antidepressant treatment induced sex-differentiated effects on T-lymphocyte proliferative capacity, with male rats showing a marked enhancement of the proliferation index and females the exact opposite. CMS application alone had
no effect on stimulated splenocyte proliferation on either sex, and chronic clomipramine administration in male and female CMS-treated rats did not further induce proliferation (Fig. 1b).

**NK cell cytotoxicity**

NK cytotoxicity was sexually dimorphic under basal conditions, with the cytolytic responses of females being more than 2-fold that of males. Chronic antidepressant treatment significantly suppressed NK cell responses only in female rats. Following CMS application, NK cytotoxic activity presented sex-related patterns with female responses being markedly suppressed and male responses being enhanced. In both male and female CMS-treated rats, chronic antidepressant treatment suppressed NK cytolytic responses. As a result, NK cytotoxicity of CMS-treated male rats returned to basal levels, in contrast to their female counterparts whose NK cytotoxic responses were further suppressed (Fig. 1c).

**LAK cell cytolytic responses**

The cytotoxic capacity of LAK cells was also sexually dimorphic under basal conditions, with females showing an advantageous response. Chronic clomipramine treatment enhanced LAK cytotoxicity in males, but had no effect in female rats. Following CMS application, LAK cytotoxic activity was negatively affected only in female rats. In the latter, clomipramine treatment acted synergistically with CMS application favouring the suppression of LAK cytotoxicity, while in male rats CMS reduced the immunoenhancing properties of chronic antidepressant treatment (Fig. 1d).

**Correlations of thymic monoamine concentrations with cell-mediated immunity levels**

Correlation analysis was undertaken separately in pooled data from Veh-treated and clomipramine-treated rats without considering the factor of sex, since inter-sex correlations were not statistically significant. Similarly, no relevant associations were revealed regarding thymic 5-HT under Veh conditions, but following chronic antidepressant treatment 5-HT appeared to be positively correlated with LAK cytotoxicity (Fig. 2d,e). In Veh-treated rats, thymic NA concentrations were negatively correlated with LAK cytotoxicity and IL-2 stimulated proliferation. In support of this finding, it has been shown that thymic NA increases with age (Cavallotti et al. 1999) in parallel with age-associated immunosenescence. Clomipramine treatment eliminated this negative influence. Thymic DA was negatively correlated with LAK cytotoxicity under both Veh and clomipramine conditions (Fig. 2d).

**Discussion**

In the present study we investigated whether cellular immunoreactivity and thymic monoamine profile are differentially affected between male and female rats in the CMS model of depression, and how these parameters are influenced upon chronic treatment with the tricyclic antidepressant clomipramine.

Monoamines have long been identified as being synthesized, released, and reacting with multiple cellular components of the immune system, including thymocytes and T-lymphocytes (Leposavic et al. 2008). However, their potential immunomodulatory role still remains elusive. In accord with data from Filipovic et al. (2008), our results confirmed that basal NA and DA thymic levels are sexually dimorphic (Fig. 2a,b). CMS application did not alter NA levels in either sex, but DA levels were augmented in female rats whereas chronic clomipramine treatment elevated thymic NA levels only in male rats. Basal levels of 5-HT, a monoamine recently detected intracellularly in thymocytes (Pallinger & Csaba, 2007), did not differ between sexes and chronic clomipramine treatment resulted in its elevation (Fig. 2c). However, this 5-HT-enhancing drug effect was diminished in CMS groups, underlining the crucial role of chronic stress in the harnessing of thymic 5-HT content. Our study revealed for the first time that chronic stress and clomipramine treatment affect thymic monoamine levels in a sex-dependent manner and in a drug- and stress-related context; however, more studies are needed in order to elucidate the mechanisms underlying our observations.

Our results regarding thymus weight indicate a sex-specific thymosuppressive effect of chronic antidepressant treatment specifically on female rats, irrespective of stress application. This finding is of prime interest since chronic antidepressant treatment has been associated with a protective or even restorative effect on thymic mass when chronic stress regimens are applied in male rodents (Freire-Garabal et al. 1997; Kioukia-Fougia et al. 2002). Thymectomy has been reported to decrease catecholamine levels and increase serotonergic neurotransmission in limbic brain regions (Song et al. 1997), suggesting that stress- or drug-induced dysregulations of the function of the thymus could also trigger a wide spectrum of neurochemical alterations in regions implicated in the
pathophysiology of MD (Song, 2002), apart from orchestrating the imbalance between humoral and cellular immune functions.

Sex differences regarding the immune status are conventionally attributed to gonadal steroids which also control cytokine secretion (De Leon-Nava et al. 2009). In our study, basal levels of NK and LAK cytotoxicity differed between male and female rats, while this was not the case for T-cell proliferation. In agreement with our results, in another study, male and female rats were characterized by comparable basal lymphocyte proliferative potential (Stefanski & Gruner, 2006). Surprisingly, NK cytolytic activity was enhanced in male rats following CMS application, whereas in other studies CMS has been associated with impaired NK-cell activity (Kubera et al. 1998). Most importantly, CMS and clomipramine treatment exerted sexually dimorphic effects on NK and LAK cytotoxic responses, with both functionalities being highly suppressed in females.

As revealed by present correlation analyses the status quo of thymic monoamines may be associated with the orchestration of cellular immune responses after adulthood. For instance, in our experimental setup, clomipramine treatment renders thymic 5-HT a positive modulator of LAK cytotoxicity. Since a significant number of LAK cells comprise of activated T-cells and 5-HT is a prominent selective T-cell immunomodulator (Frick et al. 2009), we could suggest that putative 5-HT-mediated intrathymic events (i.e. differentiation and/or a shifted cytokine secretion profile by CD4+ T-cells), could influence peripheral immunoreactivity. Nevertheless, the cellular and molecular mechanisms underlying regulation of thymopoiesis by monoamines have not yet been elucidated (Leposavic et al. 2008). It should also be noted that antidepressant drugs may influence immune functions by acting directly on lymphocytes or by affecting monoamine as well as hormonal concentrations at ‘secondary sites’, such as the spleen, which receives rich noradrenergic innervation, or blood.

Overall, cellular immunoreactivity was compromised in female rats treated chronically with clomipramine compared to their male counterparts. However, the immune effects induced by antidepressant treatment in control non-stressed rats were not always in line with those induced upon establishment of the depressive substrate, and this should be seriously considered in studies implementing chronic antidepressant administration in non-stressed rodents for the exploration of neuropharmacological phenomena. Our data complement recent reports suggesting that antidepressants prescribed to individuals suffering from pre-existing inflammatory disorders may potentiate the inflammatory state via enhancing T-cell proliferation and pro-inflammatory cytokine expression (Frick et al. 2009). Moreover, sex differences in pharmacokinetics/pharmacodynamics following antidepressant treatment may differentiate the cellular immune response between the two sexes. Thus, our results could assist the advantageous responsivity/tolerability that men present upon treatment with tricyclic antidepressants vs. selective serotonin reuptake inhibitors (SSRIs), where they are outperformed by women (Kornstein et al. 2000).

In conclusion, apart from intriguing sex differences observed at baseline, our study provided two major findings: (1) antidepressant treatment alters the monoamine profile of the thymus in a sex-dependent manner and (2) CMS application and chronic treatment with clomipramine exert sex-related effects in cellular immunoreactivity, with females presenting a relatively immunosuppressed phenotype compared to male rats. Thymic monoamine alterations being associated with functional measures of cellular immunity are suggestive of a thymus-dependent pathway by which antidepressants could affect cell-mediated immunity. To the best of our knowledge, this is the first study reporting sex differences in immunomodulation in the CMS model of depression in the prism of chronic antidepressant treatment. However, future in vivo (in other species/strains or stress regimens) and in vitro (cell responsiveness and hormone determinations) studies are needed in order for other pathways of the neuroimmune circuitry to be unravelled in the CMS or other models of depression.

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Statement of Interest
None.

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