Effects of Escitalopram on a Rat Model of Persistent Stress-Altered Hedonic Activities: Towards a New Understanding of Stress and Depression

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Abstract

Chronic mild stress (CMS) paradigm is a model to simulate clinical depression induced by long-term environmental stress. The present study investigated the effects of escitalopram, a specific serotonin reuptake inhibitor (SSRI), on depression-like activities in adult (18 week-old) Sprague-Dawley (SD) rats that underwent a total 8-week CMS. Body weight, locomotor activity and sucrose consumption of the rats were measured under CMS paradigm and following escitalopram treatment. The plasma level of corticosterone was also measured at the end of the experiment. Our results revealed that the CMS program reduced the body weight, but not the locomotor activity of the rats. Adult SD rats consumed less sucrose solution under CMS. However, chronic escitalopram regime (10 mg/kg/day for 4 weeks) appeared not helpful in reversing this CMS effect and, if any, the drug exaggerated anxiety profile of the animals. Unexpectedly, the stressed rats exhibited higher sucrose consumption than non-stressed rats after receiving repeated saline injections. Further, the stressed rats were found to have a higher plasma level of corticosterone after escitalopram treatment. Our results provide an example of the possibility that previously stressed individuals may develop an anti-depression ability that lessens the benefits of intervention with antidepressants. Finally, a separate group of rats that entered the CMS program at 10 week-old were used to examine possible effects of aging to interpret the stress coping ability observed in the 18 week-old rats. The younger rats developed less anti-anhedonia effects under repeated saline injections. The data of the present study provide a different perspective on stress-induced depression and possible interaction with antidepressants.

Key Words: anhedonia, antidepressant, chronic mild stress, depression, escitalopram, stress

Introduction

Stress is closely related to mental dysfunctions in the modern society. Persistent stress can induce depression (18). It is, therefore, not surprising that for use as a preclinical approach, the rat model of chronic mild stress (CMS) has been used extensively in exploring the environment-induced psychopathologies of depression (23). On the other hand, evidence also shows that individuals under chronic stress may
develop anti-depression abilities (7), raising a different interpretation of stress-depression relationship from the perspective of the individual’s reaction to stress. This is particularly important in terms of the appropriateness of employing antidepressants in the stress-related mood dysfunctions, given the evidence that the reported therapeutic effects of selective serotoninergic reuptake inhibitors (SSRIs) have been inconsistent in the treatment of stress-combined mood disorders (31).

Loss of interest, or namely anhedonia, is one of cardinal depressive symptoms. Ability of reversing anhedonia symptom is considered an index for antidepressive effects (21). Previous evidence demonstrated that rodent CMS paradigm had a good face validity of anhedonia, i.e. reduced responsiveness to reward, by exhibiting a lower sucrose intake in the sucrose consumption test (14, 28). However, certain aspects of using the CMS paradigm are necessary to be revisited as laboratory protocols may determine the outcome evaluation of the CMS effects and also to what degree antidepressants can reverse these effects (36). First, rats prior to entering into the CMS paradigm are in general housed singly, which may confound the results obtained, since social isolation itself induces depression-like activities (6, 10, 32, 39). Second, the relatively short duration of two weeks of CMS before the treatment regime, or the drug intervention launched at the same time of CMS, apparently does not parallel clinical situation in which patients usually suffer from the stressors long before they can be treated. Third, the age of rats entering the CMS program is crucial for mediating the drug effects (15); however, the majority of evidences so far obtained were from rats entering the paradigm at their preadolescent age (30, 32, 34, 39). Last but not the least, since rat strain is a determinant for sensitivity of the CMS model (2), it is interesting to have an idea of what would happen in rats of lower CMS sensitivity, yet which would endure a longer duration of CMS.

The present study aimed to clarify the above issues by examining the CMS effects on locomotor activity and sucrose consumption in a rat strain with a modest sensitivity to anhedonia (i.e. SD strain, see 37). Rats at their adult age were housed in groups and entered the CMS paradigm for 8 weeks; in the last 4 weeks, the rats were treated with escitalopram, a quick onset selective serotonin reuptake inhibitor (SSRI). Finally, the level of plasma corticosterone of the rats was measured at the end of the experiment to corroborate with the behavioral data. Our results may provide a different and new perspective for stress-induced depression and interactions of such depression with antidepressants.

Materials and Methods

**Animals**

Adult male Sprague-Dawley (SD) rats (BioLASCO, Taiwan) arrived at the housing room at 14 weeks of age, and weighing 250-300 g. The animals were housed at a constant cage temperature (22 ± 1°C) and humidity (40%-70%). The animals were allowed to adapt for 2 weeks to the new environment before any experiments were performed. Rats were housed in groups of three and were kept under regular light-dark conditions (light on at 07:00 AM and off at 7:00 PM), with food and water available ad libitum, except during behavioral testing. Considering the diurnal variation in sensitivity to CMS, the CMS effects on sucrose consumption were tested at the start of the dark phase (8). The study was approved by the Institutional Animal Care and Use Committee of National Defense Medical Center.

**Experimental Design**

Rats at 18 weeks of age (14 weeks at the time of arrival, 2 weeks for adaptation, and 2 weeks for sucrose training) were randomly assigned to either the non-stressed or the stressed group (i.e. rats in CMS) for 8 weeks. The escitalopram regime was introduced at Week 5 and maintained for 4 more weeks. For both groups, body weight, locomotor activity and sucrose consumption were measured during the treatment regime. A separate group of rats that entered the CMS program at 10 weeks of age were used to validate the aging effects. They received the same CMS program and were tested for their sucrose consumption after 1 week of saline injections.

**CMS**

Various unpredictable stressors were presented for 20 h per day (from 1 PM to 9 AM of the next day) for 8 weeks. The stressors included food and/or water deprivation, housing in a cage tilted at 45° with overnight illumination, housing in a cage with soiled and wet bedding, and individual housing in different cages with odors. For each day, a single stressor was selected in a random and non-repeated base. For control, non-stressed rats were used and were placed in the hoarding room without any disturbance.

**Locomotor Activity**

The locomotor activity test was identical to the procedure previously used by our team (22). In brief, the total distance traveled was measured for 30 min and recorded by a programmed microcomputer (MED Associates, Albans, VT, USA). The system included 4 plexiglas chambers (43 × 43 × 30 cm³) equipped...
with an I/R array of 16 photodetectors and corresponding light sources that emitted photo beams 3 cm apart and 4.5 cm above the chamber floor. Travel distance was recorded constantly at the assigned intervals and was controlled by Med-Associates software.

Sucrose Consumption Test

The protocol of sucrose consumption test was adapted from Wu and Wang (37). A 2% sucrose solution was used to serve as a more potent reinforcer (1, 26) with a stable pattern of intake (36). Specifically, rats learned that sucrose solution was the only available source of intake after 18-h deprivation of food and water. When testing, rats consumed the sucrose solution at the start of the dark phase for 60 min. The amount of consumed sucrose was obtained by measuring the weight of the bottle after testing.

Drug

Escitalopram (sponsored by Lundbeck, Denmark) was dissolved in 0.9% saline solution and was administered intraperitoneally in a volume of 0.1 ml/100 g body weight. A dose of 10 mg/kg/day was selected according to a previous study (32).

Plasma Corticosterone

Rats were sacrificed at the end of the experiment and blood samples were extracted and centrifuged. Plasma corticosterone was measured using an enzyme-linked immunosorbent assay (ELISA) kit following the manufacturer’s instructions (Cayman Chemical Company, Ann Arbor, MI, USA). In brief, AChE tracer and EIA antiserum were added to the wells of the plate and incubated for 2 h at room temperature on an orbital shaker. Ellman’s reagent was then added in the dark for 90 min. The value of corticosterone was measured using an ELISA reader at a wavelength of 412 nm.

Data Analysis

Statistical analyses of the present study were conducted by SPSS 16.0 for Windows (Chicago, IL, USA). Data were analyzed by a multiple-way of analysis of variance (ANOVA) in which STRESS (non-stressed vs. stressed) and TREATMENT (saline vs. escitalopram) were between-subject factors. TIME was employed as a within-subject factor, i.e., repeated measurements. Tukey HSD method was used for post hoc comparison. Student’s t test was also used for further analysis where appropriate. The significance of probability level was set at 0.05.

Results

Regarding body weight, rats in the non-stressed group gradually became heavier than those in the CMS group, which was evidenced by the significant effects of STRESS \( [F(1,22) = 13.4, P < 0.01] \), TIME \( [F(4,88) = 60.8, P < 0.01] \), and STRESS × TIME \( [F(4,88) = 56.7, P < 0.01] \). Analysis revealed that simple main effects existed for STRESS at Week 3 \( [F(1,110) = 43.2, P < 0.01] \) and Week 4 \( [F(1,110) = 40.9, P < 0.01] \), and TIME in the non-stressed condition \( [F(4,88) = 92.8, P < 0.01] \), contributed from the bodyweight change at Week 3 and Week 4 (Fig. 1, upper-left panel). The CMS-induced bodyweight decrease was restored gradually, as evidenced by a greater weight gain along the treatment time \( [F(1,21) = 96.6, P < 0.01, \text{ Week 1}; F(1,21) = 5.82, P < 0.05, \text{ Week 2}; F(1,21) = 40.4, P < 0.01, \text{ Week 3}] \), and an effect of TREATMENT × STRESS \( [F(1,21) = 5.23, P < 0.05] \) at Week 3. Further analysis revealed effects for STRESS in rats that received saline \( [F(1,10) = 20.4, P < 0.01] \) or escitalopram \( [F(1,10) = 19.0, P < 0.01] \) (Fig. 1, bar panels).

In the locomotion test, both the non-stressed and the stressed rats presented a gradual decrease over TIME in their travel distance at baseline \( [F(11,242) = 27.9, P < 0.01] \) and after 4 weeks of CMS \( [F(11,242) = 69.0, P < 0.01] \). Furthermore, there was a main effect of TREATMENT \( [F(1,21) = 13.8, P < 0.01] \), as both non-stressed and stressed rats traveled longer after 2 weeks of being treated with escitalopram (Fig. 2).

For the sucrose consumption test, 4 weeks of CMS decreased the amounts of consumption, as evidenced by a significant STRESS × TIME \( [F(1,22) = 4.84, P < 0.05] \) and simple main effects for STRESS in the condition after 4 weeks of CMS \( [F(1,44) = 12.4, P < 0.01] \), and for TIME in rats assigned to the CMS group \( [F(1,22) = 25.7, P < 0.01] \). Post hoc comparisons confirmed a strong consumption difference between stressed and non-stressed rats \( (P < 0.01) \) (Fig. 3, left panel) and the proportion of CMS rats with reduced sucrose intake was 75%. For sucrose consumption 1 week after drug treatment, significant effects of STRESS \( [F(1,21) = 11.1, P < 0.01] \) and TREATMENT × STRESS \( [F(1,21) = 12.0, P < 0.01] \) were observed. Further analysis revealed that simple main effects existed for TREATMENT in the non-stressed rats \( [F(1,21) = 4.47, P < 0.05] \), and for STRESS in the rats receiving saline \( [F(1,21) = 21.8, P < 0.01] \) (Fig. 3, middle panel), indicating the existence of anhedonia in both non-stressed rats under escitalopram treatment and stressed rats under repeated saline injections. Sucrose consumptions before and after treatment were also compared. Non-stressed rats consumed less sucrose under saline injections \( [t(10) = 3.6, P < 0.01] \), whereas stressed rats consumed more under escitalopram treatment \( [t(10) = 8.2, P < 0.01] \) (Fig. 3, left and middle panels).
Fig. 1. Effects of CMS and escitalopram (10 mg/kg/d for 21 d) on the bodyweight of stressed and non-stressed rats. The percentage (%) of weight gained across the treatments was compared (n = 12 for each group, upper left panel; n = 6 for each subgroup, bar panels). Values are presented as mean ± SEM; # indicates $P < 0.05$ vs. saline condition; ** indicates $P < 0.01$ vs. non-stressed rats.

Fig. 2. Effects of escitalopram on locomotor activity in stressed and non-stressed rats. Data were collected for 60 min and presented in centimeters of the travel distance in the conditions of baseline, 4 weeks of CMS (for stressed and non-stressed rats, n = 12 for each group, see 2 left panels), and 2 weeks after treatment (saline or 10 mg/kg/d of escitalopram, n = 6 for each subgroup; for comparison, layout with bars, see the right panel). The CMS program was still ongoing during the drug treatment stage. Values are presented as mean ± SEM; ## indicates $P < 0.01$ vs. saline condition.
For sucrose consumption 3 weeks after drug treatment, no effect of STRESS and TREATMENT × STRESS were observed. However, the stressed rats still consumed a greater amount of sucrose when injected with saline \([t(10) = 1.9, P < 0.05]\) (Fig. 3, right panel).

A separate experiment with rats entering the CMS program at 10 weeks old demonstrated that the amount of sucrose consumption was significantly lower in the stressed rats after 4 weeks \([6.8 \pm 1.2 \text{ vs. } 11.7 \pm 1.3, \text{ for stressed and non-stressed rats, respectively, } t(18) = 2.49, P < 0.05]\). However, the 1-week saline injection did not increase consumption for younger rats \([12.0 \pm 1.5 \text{ vs. } 13.7 \pm 1.1, \text{ for stressed and non-stressed rats, respectively, } t(18) = 0.36, \text{ not significant}]\) (Fig. 4).

For corticosterone concentration, a significant effect of STRESS \([F(1,21) = 6.94, P < 0.05]\) and TREATMENT × STRESS \([F(1,21) = 8.60, P < 0.01]\) was observed. Further analysis revealed that simple main effects existed for TREATMENT in the stressed rats \([F(1,21) = 9.78, P < 0.01]\) (Fig. 5).
Discussion

The present study demonstrated that adult SD rats consumed less sucrose solution under CMS. However, chronic escitalopram regime appeared not helpful in reversing the CMS effect and, if any, the drug unexpectedly exaggerated the anxiety profile of the animals. These findings are discussed below.

Consistent with a previous report (35), in the present study escitalopram increased the travel distance in the locomotion test for all groups of rats. This observation indicates that the change of locomotor activity is nonspecific (24), and a stimulant effect of the drug, if exists, affects both the stressed and the non-stressed rats. Sucrose consumption or preference, however, is a more specific index of hedonic level. Changes of this figure are useful for modeling depression-like symptoms in terms of face validity, and if the reduced sucrose consumption/preference can be reserved by antidepressants, it is also useful for predictive validity (20). The present study demonstrated that although CMS validated its role in anhedonia-like symptoms of depression, escitalopram failed to reverse this CMS effect. For sucrose consumption, although bodyweight changes may not reflect the degree of depression (9), it may influence the consumption result and should be taken into account. We calculated the amount of consumed sucrose per rat and per gram bodyweight, and both calculations concluded that that the chronic regime of escitalopram appeared ineffective in treating CMS rats with no hedonic deficit.

The underlying mechanisms of the above observations may be complicated; however, the following factors should be considered. First, it was unlikely that the rats disliked the taste, because the 2% sucrose concentration used was far from the aversive range due to over-sweetness, which is 7%-15% (26). Second, the high level of sucrose consumption in the CMS-SAL rats made it difficult for the therapeutic effect of escitalopram to be prominent. Third, since rats in our study were housed in groups, the CMS effects were unlikely confounded by isolation-induced depression (11). This is particularly important for interpreting the effects of escitalopram, given that the effects of SSRIs interact considerably with social isolation (4, 29).

On the other hand, our data seem to raise a possibility that individuals undergoing persistent stress might develop an ability to cope with the depression, given the evidence that the stress of repeated injections may induce depression (19). In other words, persistent stress itself may resemble the chronic antidepressant treatment (7), which can be also interpreted as a stress-coping over-intake/consumption (13). This is plausible because the phenomenon was more prominent after one week rather than three weeks of injections, the stress-coping consuming appeared degraded as the time elapsed.

As mentioned in the Introduction, rat strain and the protocol used can influence the outcome evaluation of CMS. This can be exemplified by a recent work in which Papp and colleagues demonstrated that citalopram, the original form of escitalopram, reversed the CMS-decreased sucrose consumption (27) by using the CMS-sensitive Wistar rat strain, with a shorter CMS duration of two weeks before the drug intervention, and a situation of singly housing which may yield depression-like activities, to a degree may have confounded the results (6, 10, 32, 39). In the present study, CMS successfully reduced the sucrose consumption in the SD rats, but the sensitivity was not as well as the Wistar strain for contrasting effects of the drug. Thus, Wu and Wang et al. (37) conclude similarly that antidepressants are probably not helpful for CMS-induced depression in SD rats. Our data are clinically relevant because they may refer to a population of patients who are relatively insensitive to persistent and unpredictable stress (here, as the CMS program), and who may develop an anti-stress ability under an add-on predicted stress (here, as repeated injections). For these patients, antidepressant benefits may not be as good as those stress-sensitive ones. The unexpected effect of escitalopram is not a unique occurrence. In fact, citalopram, the racemic form of escitalopram, has been found to be ineffective in treating stressed-mice with less hedonic deficit (33).

Aging may also influence the ability to cope with stress. Herrera-Pérez et al. (16) reported that age-dependent testosterone may play a role in stress-induced depression in which orchidectomized rats of 3-5 months, corresponding to 18 week-old rats in our experiments, did not increase their vulnerability to develop anhedonia. The observation appeared to justify the anti-stress ability of our adult rats. Note that the rats entering the CMS program in the present study were at their adult age, and were older than rats at their preadolescent age reported in other CMS studies (3, 30, 34, 39). To clarify whether the CMS effects obtained in our study were relevant to age, a separate experiment was performed using younger rats that entered the CMS program at their adolescent age (i.e., at 10 weeks old). The results revealed that these younger rats developed less anti-anhedonia effects, supporting our hypothesis that stress-induced effects vary with age (5, 15).

Plasma cortisol levels in general represent the function of the hypothalamic-pituitary-adrenal axis and correlate with stress and depression (12, 25, 40). A previously study demonstrated that SSRI antidepressants could reverse the CMS-increased plasma level of corticosterone in rats if they were sensitive
to the CMS program (38). Unexpectedly, our behavioral data showed that chronic escitalopram raised the level of plasma corticosterone in CMS rats. Mechanisms leading to such observed increased corticosterone levels can be complex. It is noted that citalopram, the original form of the drug, when chronically administered renders a similar elevation of the corticosterone levels in stressful SD rats (17).

Taken together, the present study demonstrated that chronic escitalopram regime might not be helpful in reversing the CMS-induced anhedonia-like symptoms and even unexpectedly exaggerate the anxiety profile of the treated animals. We provide here a different perspective on stress-induced depression and its interaction with antidepressants. However, some limitations of the present study should be addressed.

First, we did not have the data showing the effects of escitalopram in the young rats and, thus, there is a lack of comparison versus the 18 week-old rats. Second, a dosing pattern for escitalopram is necessary for future studies to validate this paradoxical drug effects. Third, a concomitant no-injection control group should be included to validate injection-induced mood changes. Finally, other features of depressive-like behavior, such as increased exploration of novelty are best included when evaluating the stress-induced depression-like behaviors in rats.

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References


