A Possible Potentiating Antidepressant Effect of Venlafaxine by Recombinant Rat Leptin in a Rat Model of Chronic Mild Stress

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Abstract: The present study was designed to investigate the possible changes in forced swimming test (FST), prefrontal cortical glutamate and gamma amino-butyric acid (GABA) contents by leptin and/or venlafaxine in chronic mild stress (CMS)-induced anhedonia in male albino rats. They were divided into 5 groups: the first group was not exposed to CMS, the second group received normal saline with exposure to CMS, the third group received leptin 1 mg/kg/day intraperitoneally (ip) for 3 weeks after CMS induced anhedonia was assesed by sucrose consumption test, the fourth group received venlafaxine 8 mg/kg/day ip for 3 weeks after CMS protocol, and the fifth group was received both medications for 3 weeks. Leptin and/or venlafaxine restored the changes in sucrose consumption test, behavioural assessment by forced swimming test (FST) as well as prefrontal cortical GABA and glutamate contents in the control stressed group. Furthermore, combination of both treatments seems to be more efficacious than venlafaxine alone in these parameters. In conclusion,these results showed a potential antidepressant role of leptin and beneficial therapeutic interaction with venlafaxine by affecting the GABA and glutamate level in prefrontal cortex. These actions could make leptin a potentially valuable drug for the treatment of depression.

Keywords: Chronic mild stress [CMS], prefrontal cortex, leptin, venlafaxine, forced swimming test, albino rats.

1. INTRODUCTION

Depression is the most common mental disorder with prevalence rate of about 20% of the population worldwide. It is often associated with alteration of neurochemical markers [1]. Hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis is one of the key biological abnormalities in 30–50% of depressed subjects [2]. Abnormalities in glutamate and gamma-aminobutyric acid (GABA) signal transmission had been postulated to play a role in depression [3]. Antidepressants exert their therapeutic effects *via* increasing corticolimbic monoamines [4-5]. The limited efficacy of antidepressant, delayed onset of action, and undesirable side effects lead to ongoing efforts to identify improved therapeutic agents. Leptin, anti-obesity hormone, is a hormone secreted from adipocytes and enters the brain by a saturable transport mechanism. By binding to its receptors in the hypothalamus, it act as negative feedback adiposity signals [6]. Some studies suggested that leptin may be a novel antidepressant [7], however further investigations are needed to confirm the leptin's antidepressant efficacy and its possible interaction with antidepressant drugs and to identify its possible mechanism. Chronic stress is considered as a predisposing factor in the onset of depression in humans. Rats exposed to chronic unpredictable stress showed decrease in plasma leptin independent of body weight alteration [8]. Leptin treatment reduced both dopamine release and tyrosine hydroxylase concentrations in the nucleus accumbens of ob/ob rats [9]. These findings are suggestive of that leptin enhances the mesolimbic dopamine activity. However, additional studies are necessary to clarify the exact role of leptin on other neurotransmitters.

Monoaminergic theory of mood disorders has yielded a broad range of pathophysiological insights over a long time [3]. Meanwhile, the possible involvement of GABAergic system, in pathophysiology and treatment of mood disorders, is an important target of on-going psychiatric studies [10]. GABA is synthesized in a single step from its precursor glutamate by glutamic acid decarboxylase. GABA is metabolized by successive transamination and oxidation to yield succinic semialdehyde and succinic acid, respectively. As a part of the transamination reaction, a recycling system is formed in which α-ketoglutaric acid is converted to the GABA precursor glutamate by GABA-glutamic acid transaminase [11]. The cornerstone of the GABA hypothesis in bipolar disorder is that GABA provides inhibitory action to both norepinephrine [NE] and dopamine systems [12]. Although this widely expressed neurotransmitter has been thought to exert a tonic inhibitory effect on norepinephrine[NE] systems, recent data suggested that GABA may in fact facilitate NE activity [13]. There was a high evidence for depressed patients to have lower levels of GABA in their blood plasma. These low plasma levels are thought to reflect lower brain levels [14]. Accordingly, The present study was designed to assess the possible antidepressant effect of leptin and/or venlafaxine and if it is related to changes in

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glutamate and GABA concentrations in frontal cortex as an area of cognition.

2. MATERIAL AND METHODS

2.1. Animals

Sixty male albino rats weighing 180-200 g, were purchased from the National Research Center in Egypt. The rats were housed in cages under standard experimental conditions: room temperature 24 ± 1°C and 12-h light–dark cycle (lights on at 8:00 AM). Food and tap water were freely available. Rats were allowed to have seven days acclimation before any experimentation. After acclimation, rats were randomly divided into 5 groups [n in each = 12 rats].

2.2. Materials

Recombinant rat leptin [Sigma chemicals Co], Venlafaxine HCl (Wyeth-Ayerst) were purchased as powders and were dissolved in saline.

Doses: leptin and venlafaxine hydrochloride were given daily intraperitoneal (ip) as 1 mg/kg [6] and 8 mg/kg [14], respectively.

Gamma aminobutyric acid (GABA), L-glutamate and norvaline standards (Sigma chemicals Co, USA), ethanol, (HPLC grade, Merck, Germany), triethylamine (Merck, Germany), phenylisothiocyanate (PITC, Sigma chemicals Co., USA), hydrochloric acid (32%, Merck, Germany), acetonitrile (Merck, Germany), glacial acetic acid (Sigma chemicals Co., UA), sodium acetate anhydrous (Merck, Germany).

2.3. Methods

2.3.1. Chronic Mild Stress Procedures (CMS): Three-Week Application of Stressors Procedure

It was conducted according to the method of Willner *et al.* and Solberg *et al.* [15- 16]. Rats in the stressed groups were subjected to different stressors over 3 weeks without treatment in groups (2,3,4,5) to induce anhedonia in rats which simulate human depression, then groups (3,4,5) continued to expose to CMS model with treatment either with venlafaxine, leptin or both.

CMS model involved the exposure of tested albino rats to 16-h water deprivation (water bottles were removed from the cages during this time), 5 min.-tail suspension (animals were held upside down by their tail with metal tongs), one to two hours restraint (animals were placed in a 50 ml conical tube with breathing holes), 30-45 min. paired housing (the mouse was placed in the cage of another mouse of the stress group, each week the home cage mouse was alternated), soiled cage: 100 ml (16-18°C) water was poured into the cage and 5-min forced swim in cold water (16-18°C). Each week, the stressors were presented in a different order and given at different times of the day.

2.3.2. Sucrose Consumption Test

The development of anhedonia in rats was tested by sucrose consumption test. The test was carried out once every week after stress. Six hours after light out, animals were given a bottle of 2% sucrose for a 1-h period (because the pilot study revealed that rats consumed more water during their active period, thereby, enhancing the chance of seeing a difference in sucrose consumption). After 1-hour, this bottle was removed and total sucrose consumption was calculated. The stressed animals when they become anhedonic consumed less sucrose in comparison to the control group. Preliminary data have shown that control rats prefer a 2% sucrose solution over regular unsweetened water (pilot study).

2.3.3. Experimental Groups of Rats

Group 1 Control: neither exposed to CMS model nor to treatment, only ip injection of saline during the therapeutic period of treated groups.

After exposure for 3 weeks stressors, rats were divided into 5 groups (each group=12 rats) with daily administration of saline or drugs for another 3 weeks as follows:

Group 2 exposed to CMS + ip injection of saline during the therapeutic period of treated groups i.e. stressed, saline-treated group

Group 3 CMS + leptin 1 mg/kg/day ip for another 3 weeks during exposure to CMS model

Group 4 CMS + venlafaxine 8 mg/kg ip for another 3 weeks during exposure to CMS model

Group 5 CMS + leptin 1 mg/kg ip + venlafaxine 8 mg/kg ip for another 3 weeks during exposure to CMS model

2.3.4. Forced Swimming Test (FST)

At the end of the study, the FST was done to assess the immobility according to the method of Detke *et al.* [17]. It was done by placing rats into individual

glass cylinders (46 cm height , 20 cm diameter) containing 23-25°C water 30 cm deep, so that rats could not support themselves by touching the bottom with their paws.

Two training swimming sessions were conducted initially one session (15-min) of pretest followed 24 h later by a second session (5-min) of test. After each swimming session, the rats were removed from the cylinders, dried with paper towels and returned to their home cages. A single observer, who was blind to the treatment conditions, did all the behavioral scoring.

The immobility is defined as floating in water without struggling, and doing only those necessary movements to keep the head above water. For each rat, the immobility time is calculated in seconds over a period of 5 minutes.

2.3.5. Determination of Glutamate and GABA Concentrations in Homogenates of Prefrontal Cortices of Tested Rats by High Performance Liquid Chromatography

Following the behavioural test, rats were sacrificed by decapitation. The whole brain tissues were removed rapidly on an ice-plate. The tissues were washed with cold saline. The prefrontal cortex from each rat was homogenized and samples were centrifuged in a cooling (4 °C) centrifuge at 15,000 rpm for 10 minutes. The supernatant was aspirated and transferred to an Eppendorf tube. The pellet was kept at -70°C until assayed for total protein content according to the method of Bradford [18].

High performance liquid chromatography (HPLC) with pre-column phenyl-iso-thio-cyanate (PITC) derivatization was used for determination of glutamate and GABA levels in homogenates of the prefrontal cortex of the brains of rats from different groups according to the method of Gunawan *et al.* [19]. Each sample was derivatized by drying 100 µl of the aspirated supernatant in a centrivap under vacuum. The residue was dissolved in 20 µl of ethanol-water-triethylamine (2:2:1) and evaporated to dryness under vacuum. 30 µl of ethanol-water-triethylamine-phenylisothiocyanate [PITC] (7:1:1:1) was added to the residue and allowed to react for 20 min at room temperature to form the PITC derivatives of the amino acids. Excess reagent was then evaporated under vacuum. The mobile phase of HPLC consisted of solvents A & B [solvent A: 0.1 M sodium acetate buffer (pH= 5.8); solvent B: acetonitrile:water (60:40, v:v)]. A mixture of 80% solvent A and 20% solvent B was adjusted for "isocratic" HPLC separations. Flow rate was set at 0.6 ml/min. The injected sample was 20 µl. The peaks were detected at a wavelength of 254 nm. Standard curves for glutamate or GABA and norvaline were plotted using norvaline 2 nmol/20 µl as an internal standard. The ratio of the peak area of each concentration of each standard to the peak area of the internal standard was determined and entered against the concentration of the standard in a simple regression procedure.

2.4. Analysis of the Data

The data obtained are presented as mean ±SEM [Standard error of mean] and evaluated using one-way ANOVA, followed by Tukey's post hoc determination, using GraphPad Prism version 3.00 for Windows 97 (Graph Pad Software, San Diego, CA, U.S.A.).

2.5. Ethics

All procedures were in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals, as well as the guidelines of the Animal Welfare Act.

3. RESULTS

3.1. Effect of Leptin and/or Venlafaxine on Sucrose Consumption in CMS-Induced Anhedonia in Rats

Figure **1** demonstrates the reversal of anhedonia after 3 weeks of ip administration of leptin and/or venlafaxine in male albino rats continuously exposed to CMS protocol. Sucrose consumption in mL of the different groups was calculated. In comparison to the control non stressed group, control stressed group was associated with a significant ($p<0.01$) decrease in sucrose consumption by 84.33 %. This decrease was reversed in the leptin, venlafaxine and both-treated to be -35.34%,-10.64% and +13.25% respectively as compared to the control group level. Venlafaxine and leptin were statistically more effective ($p<0.01$) than either of the medications alone.

3.2. Effect of Leptin and/or Venlafaxine on the Forced Swimming Test (FST)

Reduction of immobility time (in the FST) was observed after treatment of rats suffering from CMS with either leptin and/or venlafaxine (see Table **1**). Combination of both medications caused statistically significant reduction in the immobility time more than each one alone (Table **1**).

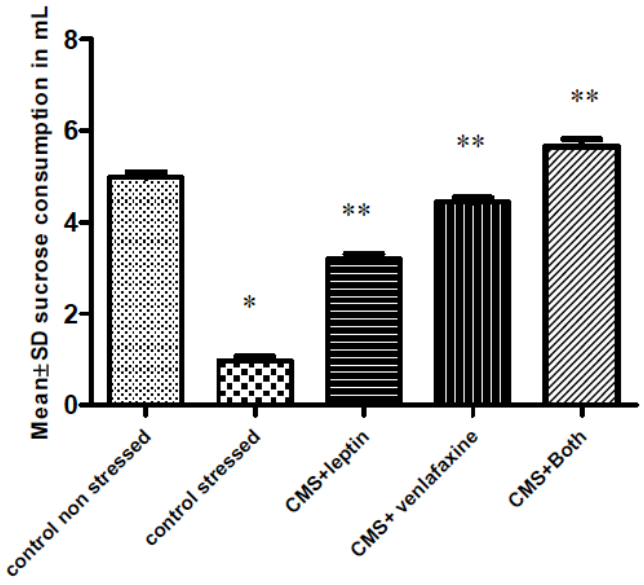

Figure 1: Influence of exposure to chronic mild stress (CMS) on sucrose consumption in male albino rats of the different groups; control saline-treated, chronic stress -with and without treatment. Data are means± SEM from 12 animals per group.

*p<0.01= significant decrease vs saline control group 1.

** p<0.01 = significant increase versus control stressed group 2.

3.3. Effect of Leptin and/or Venlafaxine on the Prefrontal Cortical Glutamate Level of CMS-Induced Anhedonia in Rats

Figure **2** represents the changes in glutamate concentration in the prefrontal cortex of the control non stressed, CMS, CMS+either leptin and/ or venlafaxine in male albino rats.

CMS increased significantly (p<0.01) the glutamate concentration in the prefrontal cortex. Glutamate concentration of CMS treated rats was decreased significantly (p<0.001) by leptin and/or venlafaxine. Administration of a combination of both medications was more effective than either one alone (p<0.01) in reducing glutamate level of CMS-treated rats.

3.4. Effect of Leptin and/or Venlafaxine on the Prefrontal Cortical GABA Level of CMS-Induced Anhedonia in Rats

Figure **3** depicts the significant (p<0.01) decrease in the GABA concentration in the prefrontal cortex in CMS- non treated group. GABA concentration of CMS rats was increased significantly by leptin and/or venlafaxine. Both medications was more effective than either drug alone (p<0.01) in increasing GABA level of CMS-treated rats.

3. DISCUSSION

In the present study, 3-weeks daily dose of leptin (anti-obesity hormone) and/or venlafaxine (serotonin norepinephrine reuptake inhibitor) induced a statistically significant increase in sucrose consumption in albino rats exposed to 6-weeks of CMS model that simulates human depression. They also reduced the immobility time in the FST as a screening test for antidepressant effect. Additionally, they increase the GABA and reduce the glutamate contents of the prefrontal cortex of these albino rats. The present experiment showed that, co-administration of leptin and venlafaxine induced the above changes in the tested parameters more than that induced by administration of either one alone and the difference was statistically significant. Furthermore, leptin alone was statistically efficient in improving these parameters but to a lesser extent than venlafaxine. These results are supported by findings of Lu *et al.* [6], which demonstrated that leptin is a novel antidepressant. Moreover, it could potentiate the antidepressant effect of venlafaxine, as demonstrated by results of the present study.

Previously, systemic administration of leptin ameliorated the chronic stress-induced decrease in sucrose preference and decreased the duration of immobility of both FST and tail suspension test (TST) in a dose-dependent manner [20]. Sucrose preference test is regarded as an analog of anhedonia, a key

Table 1: Changes in Immobility Time After 3 Weeks of Single Daily ip Administration of Either Paroxetine or Venlafaxine Starting from the End of the 3rd Week Up to the End of the 6th Week of Exposure to CMS Protocol to Male Albino Rats

Parameter	Control non-stressed	Control stressed	CMS+ leptin	CMS+ venlafaxine	CMS+both medications
Duration of immobility (sec.)	98.75±1.41	169.8±1.80	113.9±2.01*	93.83±1.09*	84.75±1.11**
% change from control		+ 71.95%	+15.34%	- 4.98%	- 14.18%
% change from CMS			- 32.92%	-44.74%	-50.09%

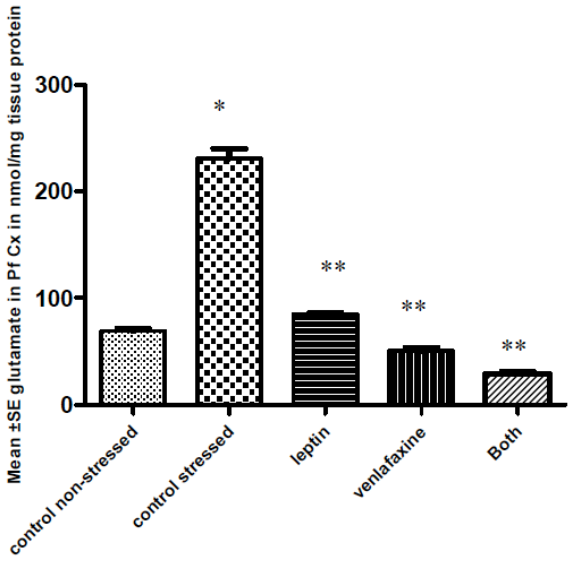

Figure 2: Influence of CMS with and without administration of either leptin or venlafaxine or both medications on glutamate in Pf Cx of male albino rats of the different groups; control stressed and non-stressed as well as CMS-treated albino rats. Data are expressed as the mean ± SEM from 12 animals per group.

*P < 0.01 significant elevation versus control-non-stressed group 1.

**P < 0.01 significant reduction versus control stressed group 2.

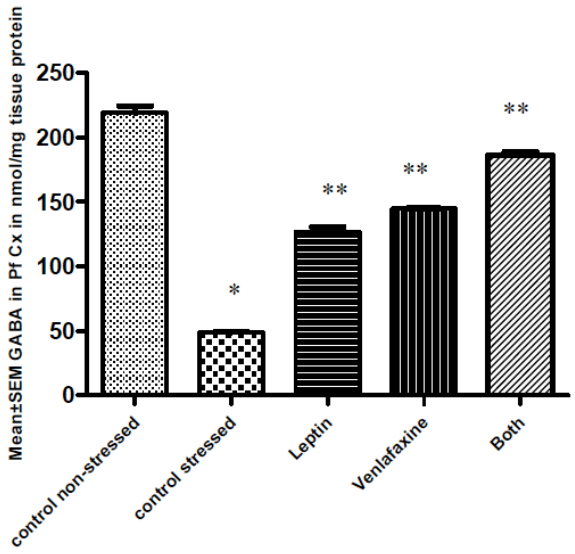

Figure 3: Influence of CMS with and without administration of either leptin or venlafaxine or both medications on GABA in Pf Cx of male albino rats of the different groups; control stressed and non-stressed as well as CMS-treated albino rats. Data are expressed as the mean ± SEM from 12 animals per group.

*P < 0.01 significant reduction versus control-non-stressed group 1.

**P < 0.01 significant elevation versus control stressed group 2.

symptom of depression in human [7, 20]. FST and TST are analogue to behavioral despair in human and have high predictive validity for antidepressant activity and have been widely used for screening antidepressant drugs [17]. Available information about leptin signaling in human depression is limited and controversial. One study described no differences in the leptin level between depressed patients and healthy controls [21]. Another studies, verified that plasma leptin levels were decreased in depressed patients with larger sample size [22-24]. As the leptin receptors are highly expressed in the limbic system, it was suspected to be the site of actions for leptin. Another study suggests that the hippocampus might be a target site for circulating leptin to exert its actions [20].

A clinical study showed that there was a low concentration of GABA in plasma and cerebrospinal fluid (CSF) of individuals with major depression [25]. In addition to that, low occipital cortical GABA concentration had also been found in depressed patients and when these patients were treated with selective serotonin reuptake inhibitor [SSRI], results revealed a normalization of its value, suggesting a role of GABA in the mechanism of antidepressant action [13]. Venlafaxine treatment was associated with increase in GABA level in prefrontal cortex of cocaine-dependant patients. Its effect was more potent than paroxetine being acting on both serotonin and norepinephrine [25]. Hypothalamic norepinephrine (NE) is involved in many of the neuroendocrine effects that are associated with leptin. An experimental study demonstrated that leptin induced changes in NE efflux through GABA. It was concluded that leptin could probably produce its central and neuroendocrine effects by modulating NE and GABA levels in the hypothalamus [26]. Zang *et al.* [27] recommended the use of cell culture systems instead of conventional animal tests for studying the pharmacological profile of new compounds that could be used as remedies. The study pointed to the expensive and obscure results that might be obtained from animal tests. It describes cell culture to be a faster and more reliable screening method for all expected biological effects of different drug candidates and phytochemicals.

4. CONCLUSION

Collectively, These results showed a potential antidepressant role of leptin and beneficial therapeutic interaction with venlafaxine by an increase in GABA and a reduction in glutamate levels in prefrontal cortex. These actions could make leptin a potentially valuable drug for the treatment of depression.

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