EFFECT OF PAROXETINE AND VENLAFAXINE ON MICE EXPOSED TO CHRONIC MILD STRESS

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ABSTRACT

The role of gamma-amino butyric acid (GABA) in mood disorders and its interaction with serotonin (5-HT) and norepinephrine (NE) systems is worthy of further study. Many studies reported that plasma GABA levels are relatively reduced in depressed patients. The present study investigated the alteration of GABA content by long-term antidepressant treatment with either paroxetine as one of the selective serotonin re-uptake inhibitors (SSRIs) or venlafaxine as a serotonin-norepinephrine re-uptake inhibitor (SNRI) in the frontal cortex (F.Cx) [as a brain region crucial for the control of emotion and cognition] obtained from male mice exposed to chronic mild stress(CMS)-induced anhedonia. The long term behavioral changes of the CMS without and with antidepressant treatment were also tested using the forced swimming test (FST). The results demonstrated the reversal of CMS-induced anhedonia after 3 weeks i.p. administration of 1 and 8 mg/kg/day paroxetine and venlafaxine, respectively. Furthermore, venlafaxine seems to be more efficacious than paroxetine in long-term behavioral changes recorded by the FST. Additionally, there was a significant (p<0.001) increase in the GABA content of the F.Cx of mice exposed to CMS-induced anhedonia. The present study suggested that GABA levels may be decreased in an animal model of depression and its reversal together with the behavior improvement by either paroxetine or venlafaxine could support the hypothesis that modification in GABAergic activity in mood disorders may complement the monoaminergic and serotonergic theories, proposing that the balance between multiple neurotransmitter systems may be altered in these disorders.

Keywords:  Paroxetine – Venlafaxine – Chronic mild stress - Mice.

INTRODUCTION

Serotonin and norepinephrine are the most studied neurotransmitters in mood disorders. Decrease in the functional activity of central serotonergic and catecholaminergic systems is probably associated in major depressive episodes (Blier & De Montigny, 1994). Applying an appropriate strategy with drugs targeting both neurotransmitter systems may improve the efficacy of the antidepressant treatment. In recent years, new antidepressant drugs, with less adverse effects than imipramine derivatives, have been developed; they selectively block either the serotonin reuptake (that is, selective serotonin reuptake inhibitors, SSRIs), or the noradrenaline (NA) transporter, or both types of receptors (that is, mixed serotonin/noradrenaline reuptake inhibitors, SNRIs, e.g. venlafaxine, duloxetine), (Artigas, 1995; Vetulani & Nalepa, 2000). SSRIs, such as paroxetine and citalopram, are effective in treating depressed patients (Delgado et al., 1992; Deakin & Dursun, 2002), however SNRIs such as venlafaxine; a compound that increased both extracellular levels of 5-HT ([5-HT]ext) and NA ([NA]ext) in the rat frontal cortex, Dawson et al., 1999; Millan et al., 2001] were suggested to be of a greater therapeutic value than SSRI (Einerson et al., 1999).

Other neurotransmitter systems may be also involved in the pathogenesis of depression. The present findings suggest that, in addition to other neurotransmitter systems and biological aberrations, GABAergic influence may be implicated in the pathogenetic mechanisms of mood disorders (Dikeos & Papadimitriou, 2003).

Gamma amino-butyriric acid (GABA) is the major inhibitory neurotransmitter in the brain and diminishes the activity of its target neurons. It is a major inhibitory neurotransmitter in the central nervous system and modulates the activity of several neurotransmitters including dopamine, serotonin, and norepinephrine. 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 (Brambilla et al., 2003).
The cornerstone of the GABA hypothesis of bipolar disorder is that GABA provides inhibitory action to both norepinephrine and dopamine systems (Goodwin & Jamison, 1990). Although this widely expressed neurotransmitter has been thought to exert a tonic inhibitory effect on NE systems, recent data (Petty et al., 1993) suggest that GABA may in fact facilitate NE activity. Petty et al. (1993) reported that plasma GABA levels are relatively reduced in depressed patients.

There is a well proven tendency for depressed and bipolar patients to have lower levels of GABA in their blood plasma. These low plasma levels are thought to reflect lower brain levels and Braverman and Pfeifer (1987), suggested using GABA to treat depression in order to bring up its plasma level. Unfortunately this theory is too simplistic and possibly dangerous.

The current theory of GABA and depression is that low plasma levels of GABA may identify an inheritable tendency for mood disorders such as depression or bipolar disease (Sanacora et al., 2002).

Hence, the role of GABA in mood disorders and its interactions with 5-HT and NE systems is worthy of further study. The present study investigated the alterations of GABA content by long-term treatment with either paroxetine (as SSRI) or venlafaxine (as SNRI) in the frontal cortex (F.Cx) [as a brain region crucial for the control of emotion and cognition] obtained from mice exposed to chronic mild stress (CMS)-induced anhedonia. The long-term behavioral changes of the CMS without and with antidepressant treatment was also tested using the forced swimming test (FST).

**MATERIALS AND METHODS**

**Drugs:**

Venlafaxine HCl (Wyeth-Ayerst) and paroxetine HCl (Glaxo SmithKline, U.K.), were dissolved in saline in a volume of 20 ml kg⁻¹. Gamma aminobutyric acid (GABA) and L-glutamate standards (Sigma chemicals Co), ethanol, [HPLC grade, MERCK], triethylamine [(TEA), MERCK], phenylisothiocyanate [PITC, Sigma chemicals Co], hydrochloric acid (32%, MERCK), acetonitrile [MERCK], glacial acetic acid (Sigma chemicals Co), sodium acetate anhydrous [MERCK].

**Animals:**

Forty-eight male albino mice, weighing 20-25 gm, were used allover experimental procedures. They were randomly allocated into 4 groups, number of animals in each group=12. Mice were allowed one week to acclimate to the surroundings before beginning any experimentation. Animals were housed in individual plastic cages. Food and tap water were available ad libitum for the duration of the experiments unless otherwise noted. Sucrose solution (2%) was available ad libitum for one week preceding the experimental procedures to allow adaptation to the taste of sucrose. The temperature was maintained at 22±2°C. The light-dark cycle (LD) was on a 12 h light/dark cycle with lights on at 06:00 a.m. and off at 06:00 p.m., unless otherwise noted during the stress procedure (6 weeks). All experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

**Experimental protocol:**

Mice were weighed and each one was placed in an individual cage. To introduce the mouse to sucrose solution and to obtain baseline data on sucrose consumption, mice were given bottles of both water and 2% sucrose. Twenty-four hours later, the bottles were removed and weighed to measure liquid intake. The water bottles were then replaced. Sucrose intake was measured again for a 1-h period. On the basis of body weight and sucrose intake (during the 24- and 1-h periods), mice were assigned to experimental or control groups (n=12 in each group). Body weight, in addition to sucrose consumption, was used to separate animals in an effort to minimize future changes in sucrose intake caused by differences in body size. Experimental animals were exposed to 6 weeks of chronic mild stress. Antidepressant-treated animals received a daily dose (i.p.) of either paroxetine or venlafaxine starting from the beginning of the 3rd week up to the end of the 6th week of CMS. The control animals were left undisturbed during the 6 weeks-period, except for scheduled daily i.p. injection of saline in the last 3 weeks simulating the test group of treated animals, in addition to cleaning, feeding and weighing procedures.

During the stress period, control and experimental animals were weighed weekly. A 1-h sucrose test was given to all animals once a week. At the end of the experiment, a forced-swim test (FST) was done to assess long-term behavioral changes of the chronic stress protocol according to Solberg et al. (1999).

**Drug administration and Forced Swimming Test (FST):**

Where indicated, mice were injected i.p. with a once daily dose of either saline (control group), paroxetine (1 mg/kg) or venlafaxine hydrochloride (8 mg/kg), all dissolved in saline in the last 3 weeks of CMS. The injected volume did not exceed 20 ml/kg body weight. The doses of paroxetine and venlafaxine were those used by David et al. (2003).

The forced swimming test (FST) is used to test the behavioral despair in rodents (Solberg et al., 1999). It can be seen as a way to measure "fighting spirit" of mice. In the first 2 min, the animal was allowed to adjust to the new conditions, then, the immobility time that alternated with conditions of enhanced motor activity was measured. Immobility time was measured with a stopwatch for the next 4 minutes (Porsolt et al., 1977). Mice were removed from their cages and placed in individual glass cylinders (diameter 15 ml) containing water at 22-24°C at a depth of
14-16 cm so that they could not escape and could not touch the bottom. The animals were placed in the cylinders for observation in a 6-min test swim. Two swimming sessions were conducted: an initial 15-min pretest followed 24 h later by a 6-min test). The duration of immobility was measured for a 6-min period. The duration of immobility during the last 4 min. of the 6 min. test was measured by 2 trained experimenters. The mouse was considered as immobile when it stopped struggling and moved only to remain floating in the water, keeping its head above water. Shorter immobility time is an indicator of the stronger antidepressant effect of the tested substance. (Urani et al., 2001).

**Chronic stress procedure:**

The chronic stress procedure was adopted from Willner et al. (1987). Solberg et al. (1999). The protocol consisted of the following stressors:

- **a**- 16-h water deprivation (water bottles were removed from cage during this time)
- **b**- 5 min.-tail suspension (animals were held upside down by their tail with metal tongs)
- **c**- 1-to2-h 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 alternated)
- **d**- Soiled cage (100 ml 16-18°C water was poured into the cage)
- **e**- 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.

**Sucrose test:**

Preliminary data have shown that mice prefer a 2% sucrose solution over regular un-sweetened water (pilot study). Once each week, animals were given bottles of both water and 2% sucrose for a 1-h period, this occurs 6 hours after lights out (because the pilot study revealed that mice consumed more water during their active period), thereby, enhancing the chance of seeing a difference in sucrose consumption. After 1-hour, both bottles were removed and total sucrose consumption in mL of the different groups (control, CMS, and venlafaxine respectivel y, to male albino mice continuously exposed to CMS protocol. Sucrose consumption in mL of the different groups (control, CMS, CMS+chronic paroxetine medication or chronic venlafaxine medication) was calculated. In comparison to the control-saline injected group, the CMS group was associated with a (-75.55%) decrease in sucrose consumption [1.11±0.067 vs 4.54±0.12 mL]. This decrease was reversed in the paroxetine and venlafaxine-treated groups to -11.23% and -4.78% respectively of the control group level, [4.03±0.035 and 4.33±0.069mL versus the control value of 4.54±0.12 mL]. mean±SD]. The effect of the two drugs was statistically significant (p<0.001).

**Quantification of the total tissue protein:**

This was done according to Bradford (1976). The aim is to relate the GABA concentration to the total tissue protein.

**Analysis of the data**

The data obtained are presented as means±SD of mean and evaluated using one-way ANOVA, followed by Bonferroni’s post hoc determination, using GraphPad Prism version 3.00 for Windows 97 (Graph Pad Software, San Diego, CA, U.S.A.).

**RESULTS**

I. Effect of paroxetine and venlafaxine on CMS-induced anhedonia in mice:

Figure (1) demonstrates the reversal of anhedonia after 3 weeks i.p. administration of 1 and 8 mg/kg/day paroxetine and venlafaxine respectively, to male albino mice continuously exposed to CMS protocol. Sucrose consumption in mL of the different groups (control, CMS, CMS+chronic paroxetine medication or chronic venlafaxine medication) was calculated. In comparison to the control-saline injected group, the CMS group was associated with a (-75.55%) decrease in sucrose consumption [1.11±0.067 vs 4.54±0.12 mL]. This decrease was reversed in the paroxetine and venlafaxine-treated groups to -11.23% and -4.78% respectively of the control group level, [4.03±0.035 and 4.33±0.069mL versus the control value of 4.54±0.12 mL]. mean±SD]. The effect of the two drugs was statistically significant (p<0.001).
II. Antidepressant effect of paroxetine and venlafaxine elicited on the forced swimming test (FST):

Reduction of immobility time (in the FST) was observed after treatment of mice suffering from CMS with either paroxetine or venlafaxine treatment (see Table 1). Venlafaxine seems to be more efficacious than paroxetine in long-term behavioral changes of the chronic stress protocol, as the reduction in the immobility time was more after treatment with venlafaxine (Table 1).

III. Effect of chronic (3 weeks) administration of paroxetine and venlafaxine on the GABA level in the frontal cortex of chronic mild stress (CMS)-exposed mice

Figure (2) represents the changes in GABA concentration in the frontal cortex (F.Cx) of the control, CMS, CMS + chronic paroxetine or venlafaxine treated mice. CMS decreased significantly (p<0.001) the GABA concentration in the frontal cortex. GABA concentration of CMS mice was increased significantly (p<0.001) by paroxetine or venlafaxine treatment.

DISCUSSION

In the present study, 3-weeks single daily dose of paroxetine (SSRI) and venlafaxine (SNRI) induced increases in the GABA content of the FCx of mice exposed to chronic mild stress-induced anhedonia. These findings were in accordance with those of David et al. (2003) using the same doses of the anti-depressant drugs (paroxetine 1mg/kg & venlafaxine 8 mg/kg). In our experimental conditions, venlafaxine and paroxetine have increased GABA level. Furthermore, venlafaxine was more efficient than paroxetine in reducing the time of immobility of mice exposed to chronic mild stress. The frontal cortex (FCx) plays a crucial role in processes involved in the control of mood, cognition and motor behavior, functions that are compromised in depression (Millan et al., 2000).

Table (1): Changes in immobility time after 3 weeks of single daily i.p. 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 mice.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Saline control</th>
<th>CMS group</th>
<th>CMS + paroxetine treatment</th>
<th>CMS + venlafaxine treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of immobility (sec.)</td>
<td>102.7±1.22</td>
<td>175.1±2.11</td>
<td>114.7±2.02*</td>
<td>97.08±1.49*</td>
</tr>
<tr>
<td>% change from control</td>
<td></td>
<td>+70.50%</td>
<td>+11.68%</td>
<td>-5.47%</td>
</tr>
<tr>
<td>% change from CMS</td>
<td></td>
<td></td>
<td>-34.5%</td>
<td>-44.56%</td>
</tr>
</tbody>
</table>

* p<0.001 compared to the CMS non-treated group of animals

Figure (1): Influence of exposure to chronic mild stress (CMS) on sucrose consumption in male albino mice of the different groups; control saline-treated, chronic stress - with and without antidepressant-treatment. Data are means± SD from 12 animals per group. *p < 0.001 vs saline control.
In our experiments CMS reduced the consumption of the sucrose solution, that was evident within 3 weeks after the beginning of stress. A 3-weeks treatment with either paroxetine or venlafaxine almost restored the consumption to values nearby the normal saline control levels. The development of an "anhedonia-like" condition has been confirmed by the forced swimming test (FST) the duration of immobility was almost completely reversed by venlafaxine treatment compared to the paroxetine-treated-group. The immobility displayed in rodents subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. The FST is a sensitive test reflecting the state of immobility simulating behavioral despair in human (Back-Rojecky et al., 2004). The prominent reduction in immobility time by venlafaxine is consistent with results reported by Connor et al. (2000).

Brambilla et al. (2003) reviewed the available literature on the preclinical and clinical studies involving GABAergic neurotransmission in mood disorders. Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter present almost exclusively in the central nervous system (CNS), distributed across almost all brain regions, and expressed in interneurons modulating local circuits. The role of GABAergic dysfunction in mood disorders was first proposed 20 years ago (Bhagwagar et al., 2004). Preclinical studies have suggested that GABA levels may be decreased in animal models of depression, and clinical studies reported low plasma and CSF GABA levels in humans suffering from mood disorders. Also, antidepressants, mood stabilizers, electroconvulsive therapy, and GABA agonists have been shown to reverse the depression-like behavior in animal models and to be effective in unipolar and bipolar patients by increasing brain GABAergic activity. The hypothesis of reduced GABAergic activity in mood disorders may complement the monoaminergic and serotonergic theories, proposing that the balance between multiple neurotransmitter systems may be altered in these disorders. However, low GABAergic cortical function may probably be a feature of a subset of mood disorder patients, representing a genetic susceptibility. Sanacora et al.(2002) showed that there was a low concentration of GABA in plasma and cerebrospinal fluid (CSF) of individuals with major depression. In addition to that, low GABA concentration, measured by proton [(1)H] magnetic resonance spectroscopy (MRS) study, has also been found in the occipital cortex of depressed subjects and when these patients were treated with SSRI, results revealed a normalization of the low GABA concentration, suggesting a role of GABA in the mechanism of antidepressant action. However, the application of serotonin or norepinephrine induced a large enhancement of the amplitude and frequency of spontaneous inhibitory post-synaptic currents (sIPSC) resulting in increase GABA release (Zhong and Yan., 2004). This is based on the fact that GABA release is dependent on this sIPSC in the prefrontal pyramidal neurons of the rat brain (Seamans et al., 2001). This fact could explain the inebable more potent effect of venlafaxine over paroxetine being acting on both serotonin and norepinephrine-reuptake inhibition. Venlafaxine treatment was associated with increase GABA level in the prefrontal cortex of cocaine-dependent subjects. This level was low in these subjects before venlafaxine treatment. This result was associated with decrease cocaine self-administration (Streeter et al., 2005). Venlafaxine and paroxetine were recently approved for the treatment of generalized anxiety.
disorder (GAD) due to their proposed GABA agonistic action and there is preliminary evidence that, like the SSRIs, the efficacy of venlafaxine may extend to other anxiety disorders. This is stated by An Audio Round Table Discussion (2005).

In conclusion, from a neurochemical and behavioral points of view, the present study pointed to a modulating role between the serotonin, norepinephrine and GABAergic systems in the CMS-exposed mice that is a model of human depression with a high degree of validity.

REFERENCES


Audio Round Table Discussion on the internet (2005). The Role of GABA In The Pathogenesis And Treatment of Anxiety And Other Neuropsychiatric Disorders. Am. Psychiatry Association


