Comparison of monoamine reuptake inhibitors for the immobility time and serotonin levels in the hippocampus and plasma of sub-chronically forced swim stressed rats

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Abstract: The current study was aimed at comparing the behavioral and biochemical (5-hydroxytryptamine and 5-hydroxyindoleacetic acid levels) effects of monoamine reuptake inhibitors (fluoxetine, venlafaxine and imipramine) in sub-chronically forced swim stressed rats. At the given doses of 10, 20 and 30 mg/kg, among aforesaid antidepressants, the imipramine treatment alone caused significant decline in the immobility time of rats (IC_{50} ~ 20 mg/kg). In the hippocampus of rats, the imipramine treatment caused significant elevation of 5-hydroxytryptamine (5-HT) whereas, the fluoxetine and venlafaxine elicited significant increase in 5-hydroxyindoleacetic acid (5-HIAA) levels. Likewise, in the plasma of rats, the imipramine treatment significantly increased the 5-HIAA levels whereas, the fluoxetine and venlafaxine treatment significantly elevate the 5-HT levels. It can therefore be inferred that the imipramine did not act like other monoamine reuptake inhibitors in biochemical study, which could possibly underlie its ability to be detected in forced swim test (behavioral study). Moreover, the re-uptake inhibition of 5-HT is not accountable for the antidepressant action exhibited in forced swim test.

Keywords: Fluoxetine; venlafaxine; imipramine; serotonin; forced swim test.

INTRODUCTION

Depression is a prevailing neuropsychiatric illness that is estimated to be third among global burden of diseases and will lead by the year 2030 (The global burden of disease, World Health Organization 2004). The journey of modern day antidepressant began with the chance discoveries of iproniazid (Delay *et al*., 1952) and imipramine (Kuhn, 1958) that led to the catecholamine hypothesis of depression (Schildkraut, 1965) i.e. reduction of catecholamines in brain is the underlying cause of depression, and antidepressants acts via reversing the levels to normal. Afterward, the role of 5-hydroxytryptamine (5-HT, also known as serotonin) in the effectiveness of imipramine was defined (Carlsson *et al*., 1968). This led to the transformation of catecholamine, to monoamine hypothesis of depression with much focused being given to the serotonin later on. Despite of the fact that presently most of the antidepressants are monoaminergic based yet it is unclear that the modulation of monoamines is their actual mode of action (Berton and Nestler, 2006).

Because of high sensitivity, specificity, versatility and cost effectiveness, High Performance Liquid Chromatography (HPLC) with electrochemical detector (ECD) is the most widely used method to measure the levels of monoamines and their metabolites in the biological samples (Bergquist *et al*., 2002). Because of simplicity, high specificity and reliability, the forced swim test (FST) is the most widely used animal model to evaluate the antidepressant activity of substances (Porsolt *et al*., 1977). Clinically, the antidepressants take several weeks to manifest their effect (Berton and Nestler, 2006) therefore, in pre-clinical studies, the stress alone or with treatment should be given for longer duration order to obtain substantial results.

Monoamine re-uptake inhibitors constitutes major portion of clinically used antidepressants including imipramine (non-selective monoamines re-uptake inhibitor), venlafaxine (serotonin noradrenaline re-uptake inhibitor) and the promising fluoxetine (selective serotonin re-uptake inhibitor) (Gartside and Cowen, 2006). Literature revealed several reports on the behavioral and biochemical effects of the aforementioned drugs however, the comparison of these effects in a single study has not been addressed before. The current study was aimed at investigating the behavioral and biochemical (serotonin and its metabolite, 5-hydroxyindoleacetic acid levels in hippocampus and plasma) effects of monoamine re-uptake inhibitors (fluoxetine, venlafaxine and imipramine) in sub-chronic (seven days) forced swim test.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (150-200 g) were obtained from the animal house facility of International Center for Chemical and Biological Sciences (ICCBS), University of Karachi. They were housed under standard environmental conditions (12 h light/dark cycle and 25 ± 1°C room

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temperature). All experiments were performed in accordance with the guidelines provided by National Institute of Health (NIH publication no. 85-23, revised 1985) for the care and use of laboratory animals, monitored by Animal Use and Care Committee of ICCBS.

Chemicals

The following chemicals were used: Adrenaline hydrochloride (ADR), ethylenediaminetetraacetic acid (EDTA) disodium salt, homovanillic acid (HVA), 5-hydroxyindole acetic acid (5HIAA), 5-hydroxytryptamine (5HT) creatinine sulfate complex, noradrenaline (NA) and sodium chloride (Sigma, USA). The 3,4 dihydroxyphenylacetic acid (DOPAC), dopamine hydrochloride (DA), hydrochloric acid and imipramine hydrochloride (Sigma-Aldrich, Germany). Fluoxetine (Prozac 20 mg capsules, Eli lilly Pakistan limited). Venlafaxine (Effexor XR 75 mg capsules, Wyeth Pakistan limited). Acetonitrile, methanol and 1-octane sulfonic acid (Fisher chemicals, UK). Sodium dihydrogen citrate anhydrous (Fluka, Germany). Deionized water (Simplicity 185 deionizer, Millipore, USA).

Forced swim test

The test was performed as described by Porsolt *et al*. (1977) with slight modification as sub-chronic forced swim test. A pre-test session was performed by placing the rats in the FST tank (glass tank of 17 × 17 × 50 cm; filled with water (25 ± 1^{o}C) level up to 17 cm) for 15 minutes. The injured animals or those displayed nose bleeding during pre-test session were excluded from the experiment. The following day, healthy animals received intra-peritoneal (*i.p.*) treatment of fluoxetine, venlafaxine or imipramine at doses of 10, 20 or 30 mg/kg, 1 hour prior to the test session of 6 minutes duration. For each animal, the immobility time (the animal is either static or made minor movements for keeping their head above water) was recorded for the last 5 minutes, providing 1 minute for acclimatization. The same procedure was repeated for consecutive seven days. All experiments were performed between 9 am to 1 pm.

Collection of blood and hippocampi

Blood was collected by decapitation and centrifuged at 3000 rpm for 10 minutes to acquire plasma which was ultra filtered (14000 *g* for 20 minutes, Ultracel YM-10, Microcon, Millipore, Cheng *et al*., 1994 and 1995) and subjected (20 µl) to HPLC for monoamine analysis. Hippocampi were quickly dissected from the brain, placed in liquid nitrogen, homogenized in perchloric acid (1 M, 100 mg/ml) and centrifuged at 14000 *g* for 20 minutes. The supernatant was filtered (0.22 µm) and subjected to HPLC for monoamine analysis.

HPLC analysis

The high performance liquid chromatography with electrochemical detector (HPLC-ECD, Shimadzu) was used to measure the levels of monoamines and their metabolites. The reversed phase nucleosil column (C18, 250/4.6mm, 5µm, Macherey Nagel) and citrate buffer (0.1 M sodium dihydrogen citrate anhydrous, 1 mM EDTA and 2.5 mM 1-octane sulfonic acid, pH 3.4) containing 10 % acetonitirile at flow rate of 0.5 ml / min were used.

STATISTICAL ANALYSIS

The immobility times obtained in the forced swim test are expressed as mean ± S.E.M (standard error mean) of seconds. The monoamines and their metabolites levels are expressed as mean ± S.E.M. of ng/g of hippocampus. Differences between means were evaluated by one-way ANOVA followed by least significant difference (LSD).

RESULTS

Effect of fluoxetine, venlafaxine and imipramine treatment on the immobility time of rats in the sub-chronic forced swim test

The monoamine reuptake inhibitors were tested at doses of 10, 20 and 30 mg/kg and compared with that of control (table 1). The fluoxetine and venlafaxine treatment did not significantly affect the immobility time of rats whereas, imipramine treatment caused a significant ($p<0.005$) dose dependent reduction of 42%, 62% and 68% (IC_{50} ~ 20 mg/kg) respectively.

Table 1: Effect of fluoxetine, venlafaxine and imipramine treatment on the immobility times of rats in sub-chronic forced swim test

Treatment	Dose (mg/kg)		
	10	20	30
Fluoxetine	207 ± 6	209 ± 6	209 ± 5
Venlafaxine	223 ± 3	219 ± 3	229 ± 2
Imipramine	127 ± 8***	83 ± 8***	70 ± 6***

Animals were treated with fluoxetine, venlafaxine or imipramine at 10, 20 or 30 mg/kg for consecutive seven days. Each day, one hour after treatment, the forced swim test was performed (6 minutes) and immobility time was recorded for five minutes. The values represent the mean immobility time ± S.E.M. (n = 6-8). Mean immobility time of control (saline treated) animals: 220 ± 4 seconds. * ($p<0.05$), ** ($p<0.01$) and *** ($p<0.005$) as compared to the control.

Effect of fluoxetine, venlafaxine and imipramine treatment on the levels of serotonin and its metabolite in the hippocampus of sub-chronically forced swim stressed rats

The fluoxetine and venlafaxine treatment did not significantly alter the 5-HT levels whereas, a significant ($p<0.005$) reduction in 5-hydroxyindoleacetic acid (5-HIAA) levels were evident (table 2). The imipramine treatment caused significant increase ($p<0.01$) of 5-HT levels at 30 mg/kg whereas; the 5-HIAA levels remained unaltered.

Effect of fluoxetine, venlafaxine and imipramine treatment on the levels of monoamines and their metabolite in the plasma of sub-chronically forced swim stressed rats

The fluoxetine and venlafaxine treatment caused significant ($p<0.005$) decline in 5-HT levels however, the 5-HIAA levels remained unchanged (table 2). The imipramine treatment did not significantly alter 5-HT levels however; the 5-HIAA levels exhibited a significant ($p<0.005$) rise at 30 mg/kg.

DISCUSSION

The current study was aimed at comparing the behavioral (forced swim test) and biochemical (serotonin and its metabolite i.e. 5-hydroxyindoleacetic acid levels) effects of monoamine re-uptake inhibitors (fluoxetine, venlafaxine and imipramine). In present study, fluoxetine treatment (10, 20 and 30 mg/kg) did not significantly alter the immobility times in forced swim test (Table-1), which is in agreement with earlier findings showing its inability to be detected in traditional forced swim test (Cryan *et al*., 2002). It is a selective serotonin reuptake inhibitor (SSRI) and inhibits the reuptake of serotonin in synapse thereby increasing its levels at the site of action. Contrarily, in the present investigation and that reported previously (Holt and Baker, 1996), fluoxetine treatment did not elicit significant change in the levels of 5-HT (table 2). Other SSRIs (paroxetine and sertraline) following 3 weeks of treatment also demonstrated similar results in the hippocampus of rats (Benmansour *et al*. 1999). Furthermore, in agreement with Holt and Baker (1996), 5-hydroxyindoleacetic acid (5-HIAA) levels showed a significant decline (20 and 30 mg/kg) which can be attributed to inhibitory action of fluoxetine on monoamine oxidase enzyme (MAO, degrades 5-HT to 5-HIAA) as has been reported earlier (Mukherjee and Yang, 1997 and 1999). Alternatively, reduction in 5-HIAA levels could be due to the blockade of serotonin transporter (SERT) leading to low availability of 5-HT to MAO enzyme for its degradation. However, further experiments are required to confirm whether the fluoxetine is an actual inhibitor of MAO. Moreover, despite of decrease in 5-HIAA levels, the 5-HT levels remained unaltered which can be attributed to homeostasis acting via inhibition of 5-HT synthesis. A decline in monoamines synthesis in rat brain, following antidepressant treatment has been observed by Briley and Moret, (1993) and Dorotea *et al*. (1996). In the plasma of sub-chronically stressed rats, the fluoxetine treatment caused significant ($p<0.005$) dose dependent reduction of 5-HT (table 2). Most of the whole blood 5-HT is present in the platelets, which, in similarity with the neurons, possesses the highly efficient re-uptake system (serotonin transporter, SERT) for transportation of 5-HT from plasma (Brenner *et al*., 2007). The platelets are considered as the peripheral model of central serotonergic transporters (SERT, Oreland and Hallman, 1989). Our data implicates that the fluoxetine blocked the SERT leading to the depletion of the peripheral serotonin stores, also reported earlier by Pigott *et al*. (1990), Ortiz and Artigas (1992) and Bianchi *et al*. (2002).

In present study, the venlafaxine treatment failed to reduce the immobility time (table 1), which is in agreement with Connor *et al*. (2000). In the hippocampus of venlafaxine treated rats (table 2), the 5-HT levels remained unchanged, while its metabolite (5-HIAA) exhibited a significant decline. These results are in agreement with Connor *et al*. (2000) and Wikell *et al*. (2001). In the plasma of sub-chronically depressed

Table 2: Effect of fluoxetine, venlafaxine and imipramine treatment on the levels of 5-hydroxytryptamine and its metabolite 5-hydroxyindoleacetic acid in the hippocampus and plasma of sub-chronically forced swim stressed rats

Treatment	Dose	Hippocampus		Plasma	
		5-HT	5-HIAA	5-HT	5-HIAA
Fluoxetine	10	274 ± 19	214 ± 37	268 ± 66***	3.7 ± 0.6
	20	202 ± 12	141 ± 17***	106 ± 11***	3.6 ± 0.7
	30	195 ± 29	143 ± 30***	87 ± 17***	3.9 ± 1.5
Venlafaxine	10	334 ± 45	179 ± 11***	301 ± 26***	2 ± 0.5
	20	317 ± 37	171 ± 14***	321 ± 30***	2.8 ± 0.9
	30	275 ± 19	156 ± 13***	313 ± 52***	3.6 ± 0.6
Imipramine	10	314 ± 14	268 ± 18	652 ± 70	3 ± 0.5
	20	273 ± 29	260 ± 37	704 ± 121	2.9 ± 0.1
	30	378 ± 37**	247 ± 28	600 ± 94	4.6 ± 0.6***
Control		261 ± 25	273 ± 19	640 ± 53	2.6 ± 0.4

Sub-chronic forced swim test was performed in the presence of monoamine reuptake inhibitors (fluoxetine, imipramine, or venlafaxine) at the doses of 10, 20 or 30 mg/kg followed by measurement of levels of 5-hydroxytryptamine (5-HT) and its metabolite (5-hydroxyindoleacetic acid) using High Performance Liquid Chromatography. The values represent the concentration (ng/g hippocampus or ng/ml plasma) as mean ± S.E.M. (n = 6 - 8). ***** ($p<0.05$), ****** ($p<0.01$) and ******* ($p<0.005$) represents comparison of treated with control (saline treated).

rodents, the venlafaxine treatment caused significant reduction of 5-HT level also reported earlier by Fontenot *et al*. (2009). Our data revealed that the venlafaxine elicited behavioral and biochemical effects were similar to those obtained in the presence of fluoxetine, which is strengthened by earlier reports (Reneric and Lucki, 1998; Cryan *et al*., 2005) exhibiting that the behavioral effect of venlafaxine was similar to fluoxetine at low doses.

In accordance with several reports (Borsini *et al*., 1989; Barros and Ferigolo, 1998; Kroczka *et al*., 2000), the tricyclic imipramine treatment significantly reduced the immobility time (IC_{50} ~ 20 mg/kg) of rats (table 1). In rat hippocampus, it caused a significant increase in 5-HT levels at 30 mg/kg (table 2). Literature also revealed its major effect on 5-HT in the hippocampus of olfactory bulbectomized rats (Xu *et al*., 2005). Notably, in agreement with Segawa and Mizuta, (1980**),** imipramine did not alter the levels of 5-HIAA and thus deviated from the common MAO inhibitor-like effect of re-uptake inhibitors (fluoxetine and venlafaxine). Additionally, reuptake inhibitors have been shown to reduce the whole blood 5-HT (Johnson and Knowles, 1981; Ortiz and Artigas, 1992; Slotkin *et al*., 1989). Contrarily, the imipramine treatment did not significantly alter the 5-HT levels (table 2). Therefore, further investigation should be aimed to confirm its antidepressant mechanism of action *in vivo* in rats.

In conclusion, the fluoxetine and venlafaxine did not manifest their antidepressant potential in FST and elicit similar effects (re-uptake inhibitor-like) on the serotonergic system in both hippocampus and plasma. However, the imipramine treatment caused different effect on serotonergic system than that exhibited by aforementioned reuptake inhibitors, which could possibly underlie its ability to be detected in FST. It can also be inferred that re-uptake inhibition of 5-HT is not accountable for the antidepressant action manifested in FST.

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