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SIMILAR PATTERN OF INHIBITION OF TRYPTOPHAN PYRROLASE ACTIVITY BY FLUOXETINE HYDROCHLORIDE IN BOTH SEXES OF RATS

MUHAMMAD AZHAR SHERKHELI AND SAMINA BANO*
Clinical Biochemistry & Psychopharmacology Research Unit
Department of Biochemistry, University of Karachi
Karachi-75270, Pakistan

ABSTRACT

Compounds targeting the individual enzymes of kynurenine-nicotinamide pathway have led to new neuropharmacological concepts and provide novel opportunities for therapeutic intervention. Tryptophan pyrrolase (Tryptophan-2, 3-dioxygenase; EC 1.13.11.11), a metalloprotein, is the first and rate limiting enzyme of the most important pathway for tryptophan metabolism via kynurenine-nicotinamide pathway in the liver and therefore plays a key role in regulating the physiological flux of tryptophan in to relevant metabolic pathways like synthesis of neurotransmitter, serotonin in the brain. Fluoxetine, a clinically proven antidepressant, have shown statistically significant inhibition of hepatic tryptophan pyrrolase activities (holo, total and apo form) in both the sexes of rats at 10 & 30 mg/kg doses. Despite elevated holo-enzyme form of tryptophan pyrrolase in female rats, we have found similar inhibition of tryptophan pyrrolase activity by fluoxetine hydrochloride in both male and female rats. The results are discussed in relation to gender differences in the enzyme activity and its possible role in patho-physiology of depression in females.

INTRODUCTION

Tryptophan pyrrolase (Tryptophan-2, 3-dioxygenase; EC 1.13.11.11), a metalloprotein containing porphyrin rings, is the first and rate limiting enzyme for hepatic tryptophan metabolism via kynurenine-nicotinamide pathway in the liver and therefore plays a key role in regulating the physiological flux of tryptophan in to relevant metabolic pathways (Ren & Correia, 2000), one of the pathway involves synthesis of neurotransmitter serotonin in the brain. The enzyme is composed of four identical subunits and in its fully assembled tetrameric form requires 2 molecules of heme (Fe+2-protoporphyrine IX) per molecule of enzyme protein for functional competence (Ren *et al.,* 1996). The active reduced form of the enzyme (holoenzyme) does not need the addition of cofactor haematin, whereas, the inducible form (apoenzyme) requires the addition of haematin for its activity. The enzyme is induced by its substrate (tryptophan) and glucocorticoids (Badawy, 1977).

Increased activity of tryptophan pyrrolase plays important role in the development of depression particularly when hypothalamic-Pituitary-adrenal (HPA) axis activity is impaired (Curzon 1988 and Nemeroff 1998). There are several investigations in which decreased plasma tryptophan concentrations in subjects with major depression have been reported (Meltzer and Lowy 1987 and Cowen *et al*., 1989). Increased catalytic activity of tryptophan pyrrolase contributes to the lower plasma tryptophan concentrations in major depression (Maes *et al*., 1987a). Female sex hormones like estrogens and progesterone have been found to play permissive

**Correspondence*

role in altering tryptophan metabolism by either enhancing or inhibiting tryptophan pyrrolase activity in different stages of reproductive life in females (Patnaik and Sarangi 1980). Depression has been found to be more prevalent in women of reproductive age (Gater *et al*., 1998; Hussain *et al*., 2000). Women, during specific periods of their reproductive life are more vulnerable to depression *e.g*. postpartum depression, premenstrual syndrome, perimenopause depression, gravidic depression, depression in response to use of some oral contraceptives and during some infertility treatment (Halbreich 1999).

Pharmacological agents targeting specific kynurenine pathway enzymes are now available to manipulate the concentration of neuroactive kynurenine intermediates in the brain. These compounds can be used to normalize kynurenine pathway, and have shown remarkable efficacy in animal models of central nervous system disorders, and offer novel therapeutic opportunities (Schwarcz and Pellicciari 2002). Fluoxetine hydrochloride is a clinically effective antidepressant; used to treat depression, obesity and obsessive-compulsive disorder (OCD). In previous studies (Bano *et al*., 1995, 1999 and Bano & Sherkheli, 2003) it has been reported that fluoxetine has inhibitory effects on tryptophan pyrrolase activity. Muck-Seler *et al*. (1996) and Tsuiki *et al*. (1995) also reported enhanced serotonin synthesis/turnover at 10mg/kg dose.

The present investigation looks in to differential effects, if any, on an important tryptophan-degrading enzyme to unfold male and female pattern of fluoxetine's mechanism of action. In view of specific physiological differences in males and females such investigations are warranted and help to understand gender based requirement of the therapy.

MATERIALS AND METHODS

***Animals & Treatment**:*

Locally bred male & female Wistar rats (150-200g body wt) were housed five per cage under natural light dark conditions at 25±2°C and were maintained on lab chow and water *ad libitum* under standard housing conditions. Fluoxetine hydrochloride was administered in doses (10 and 30 mg/kg) to test group of animals and appropriate control rats received IP injection of the vehicle [dimethylformamide (DMF) and saline at ratio of 1:3 v/v] at a dose of 2 ml/kg. Rats were killed after 2 hours by decapitation. Livers were perfused *in situ* with ice cold 0.95% NaCl. All samples were stored at -40 ºC until analysis.

Enzymic and other Determinations:

Tryptophan pyrrolase activity was determined in homogenates either in the absence (holo enzyme activity) or in the presence (total enzyme activity) of added (2µM) haematin (dissolved in 0.1M NaOH) as previously described. (Badawy and Evans, 1975; see also the complete description by Badawy 1981 and additional comments by Badawy *et al.* (1983). The apo-enzyme activity was obtained by difference (total enzyme activity - holoenzyme activity). Liver tryptophan concentrations were determined by a modification of a method of Denkla and Dewey (1967) as described by Bloxam and Warren (1974).

Chemicals and Drugs:

Fluoxetine hydrochloride salt (Prozac) was purchased from Elli Lilly & Company Indianapolis, IN. Haematin hydrochloride and L-tryptophan from Sigma Chemical Co. (St. Louis, MO); all other chemicals were of highest purity analytical grade.

STATISTICAL ANALYSIS

The data on the effects of fluoxetine on tryptophan pyrrolase activity and liver tryptophan were analyzed by two-way ANOVA (factor 1 sex, factor 2 drug, and interaction between the two factors). Individual comparison was made using Newman-Keuls Q-statistics. Differences between groups were considered significant when $P<0.05$.

RESULTS

Table-1 shows effects of fluoxetine HCl (10mg/kg IP) administration on hepatic tryptophan pyrrolase activity in male and female rats. Data analyzed by two-way ANOVA shows significant effect of sex and drug on holo-enzyme activity ($P<0.05$ and $P<0.01$ respectively). There was no significant effect of sex on apo-enzyme and total enzyme activities. The drug's effect was also significant on apo-enzyme ($P<0.01$) and total-enzyme ($P<0.01$) activities. The sex x drug interaction was non significant in all cases.

Individual comparison by Newman-Keuls Q-statistics shows significant inhibition (55%) of holo-enzyme activity in male rats. In female rats the inhibition was 54%. Apo-enzyme activity decreased in both the sexes by 55% and 76% respectively. The reduction in total-enzyme activity was 65% in male rats and 55% in female rats.

Table-2 shows effects of fluoxetine HCL (30mg/kg IP) administration on hepatic tryptophan pyrrolase activity in male and female rats. Data analyzed by two-way ANOVA show significant effect of sex and drug on holo-enzyme activity ($P<0.05$ and $P<0.01$ respectively). There was no significant effect of sex on apo-enzyme and total enzyme activities. The drug effect was also significant on apo-enzyme ($P<0.01$) and total-enzyme ($P<0.01$) activities. The sex into drug interaction was non significant in all cases.

Individual comparison by Newman-Keuls statistics shows significant inhibition (55%) of holo-enzyme activity in male rats. In female rats the inhibition was 48%. Apo-enzyme activity decreased in both the sexes by 52% and 50% respectively. The reduction in total-enzyme activity was 54% in male rats and 55% in female rats.

Table 3 shows hepatic tryptophan concentrations in male and female rats after fluoxetine HCL administration at doses of 10mg/kg and 30mg/kg. Data analyzed by two-way ANOVA show significant effect ($P<0.01$) of drug in both the sexes. There is no significant effect of sex and sex into drug interaction is also non significant.

Individual comparison by Newman-Keuls statistics show significant increases in hepatic tryptophan concentrations in male rats. The respective increases at 10 and 30mg/kg doses were 117% and 193%. Similarly in female rats there was a significant increase in hepatic tryptophan concentrations at 10mg/kg body wt (122%) and at 30mg/kg body wt. (174%) respectively.

DISCUSSION

Results in Table 1 and 2 show that there is no significant difference in inhibitory pattern of holo-tryptophan pyrrolase by fluoxetine in both the sexes i.e., the extent of inhibition is similar in male and female rats (55% and 54% respectively); however, we have found significant differences

in liver holo-enzyme activities in both the sexes of rats this may be due to differences in gender physiology (Patnaik and Sarangi, 1980). At 10-mg/kg dose the apo-enzyme activity inhibition is more in females as compared to male rats, this indicates that low dosage of the drug could be effective in the females suffering from depression. The difference seen in the inhibition in total and holo-enzyme activities at 10 and 30 mg/kg between the groups may be due to inhibition of synthesis of enzyme at high doses in female sex but this aspect needs further investigation. The significant increases in hepatic tryptophan concentrations (Table 3) are dose dependent and also indicate that at higher dose allostearic inhibition contributes to the reduced total, holo and apo-enzyme activities. The following points provide rationale support to these results.

Table 1
Effects of Fluoxetine HCl (10mg/Kg IP)
on hepatic tryptophan pyrrolase activity in male and female rats

Parameter	Tryptophan Pyrrolase Activity (μM of Kynurenine formed/h/g/wet wt of liver)				Two-way ANOVA		df (1, 16)
	Male		Female				
	Control	Drug	Control	Drug	Sex	Drug	Sex x Drug
Holo-enzyme	1.89 ± 0.13	0.85** ± 0.05	2.35† ± 0.15	1.09** ± 0.06	F=8.20	F=86.72	F=0.86
		55%		54%	*P<0.05	**P<0.01	NS
Total-enzyme	4.14 ± 0.2	1.43** ± 0.15	4.35 ± 0.2	1.94** ± 0.23	F=1.06	F=100	F=0.042
		65%		55%	NS	**P<0.01	NS
Apo-enzyme	1.99 ± 0.17	0.89** ± 0.2	2.44 ± 0.22	0.58** ± 0.15	F=0.0056	F=45.08	F=1.18
		55%		76%	NS	**P<0.01	NS

Experimental details are given in method's section. All values are mean ± SEM of five rats in each group (n=5). Treated group was administered fluoxetine (10mg/kg) dissolved in vehicle (DMF:Saline, 1:3 v/v). Control group received an equal volume of vehicle at dose of 2ml/kg. Statistical analysis was performed using TWO-WAY ANOVA followed by Neuman-Keuls test. The significance of difference is indicated by * P<0.05 and ** P<0.01 from respective controls and † P<0.05 & ‡ P<0.01 from similarly treated male rats. NS indicates the non-significant differences.

It is reported (Carlsson *et al*., 1985) in animal studies that female rats have a higher activity of serotonin synthesizing enzymes (*e.g.,* tryptophan hydroxylase), a greater storage capacity for serotonin in brain serotonergic neurons, a more pronounced serotonin behavioral syndrome in response to serotonin agonists, and higher brain and CSF levels of tryptophan, serotonin, and 5-HIAA compared to males. Significantly lower fasting plasma tryptophan levels were found in female control subjects (Maes *et al*., 1990a). Delgado *et al*. (1990) found that males maintained their plasma free and total tryptophan levels closer to baseline values in response to tryptophan

depletion. In depression, plasma total tryptophan levels tend to be more reduced in female than in male patients (Maes *et al*., 1990a). There is a significant negative correlation between self-rated depression and plasma levels of total tryptophan in females (Maes *et al*., 1990a). Depressed females show significantly higher xanthurenic acid excretion following tryptophan loading than depressed males (Maes *et al.,* 1987b). Females with major depression exhibit significantly higher L-5-HTP-induced cortisol responses than male major depressed subjects (Maes *et al.*, 1987a). Some of these gender related differences in serotonin metabolism may perhaps be explained by the fact that liver tryptophan pyrrolase activity is greater in females and that serotonin receptors appear to be estrogen sensitive (Maes *et al*., 2001).

Table 2
Effects of fluoxetine HCl (30mg/Kg IP)
on hepatic tryptophan pyrrolase activity in male and female rats

Parameter	Tryptophan Pyrrolase Activity (µM of Kynurenine formed/h/g/wet wt of liver)				Two-way ANOVA		df (1, 16)
	Male		Female				
	Control	Drug	Control	Drug	Sex	Drug	Sex x Drug
Holo-enzyme	2.16 ± 0.13	0.96** ± 0.04	2.44† ± 0.1	1.27**† ± 0.07	F=6.80	F=233.15	F=0.797
		55%		48%	*P<0.05	**P<0.01	NS
Total-enzyme	4.11 ± 0.26	1.9** ± 0.06	4.32 ± 0.15	1.95 ** ± 0.05	F=0.30	F=158.51	F=0.39
		54%		55%	NS	**P<0.01	NS
Apo-enzyme	1.60 ± 0.15	0.77** ± 0.04	1.96 ± 0.14	0.98** ± 0.1	F=3.91	F=41.72	F=0.386
		52%		50%	NS	**P<0.01	NS

Experimental details are given in method's section. All values are mean ± SEM of five rats in each group. Treated group was administered fluoxetine (30mg/kg) dissolved in vehicle (DMF: Saline, 1:3 v/v). Control group received an equal volume of vehicle at dose of 2ml/kg. Statistical analysis was performed using TWO-WAY ANOVA followed by Newman-Keuls test. The significance of difference is indicated by * P<0.05 and **P<0.01 from respective controls and † P<0.05 from similarly treated male rats. NS indicates the non-significant difference.

In addition to above points it is noted that; women tend to respond better to specific serotonin reuptake inhibitors (SSRIs), while men generally respond better to nor epinephrine tricyclic antidepressants (Halbreich, 1999). Secondly, women respond to lower dosages of antidepressants because the average women weigh less than the average man and has a lower blood volume and a higher percentage of body fat. Therefore lipophilic drugs, like fluoxetine, are stored at high concentrations and for longer periods, hence, increasing the half-life. Higher blood levels in women are also due to slower liver metabolism in women of reproductive age and lower renal clearance compared with males (Yonkers and Hamilton, 1995).

Table 3
Effects of Fluoxetine HCl (IP) on Hepatic Tryptophan Concentrations in Male and Female Rats

Fluoxetine (dose)	Liver Tryptophan (µg/g wet wt of tissue)				Two-way ANOVA		df (1, 16)
	Male		Female				
	Control	Drug	Control	Drug	Sex	Drug	Sex x Drug
10mg/kg	15.2 ± 1.1	32.97** ± 0.6	14.24 ± 0.47	31.66** ± 1.8	F=1.79	F=130.46	F=0.54
		117%		122%	NS	P<0.01	NS
30mg/kg	9.96 ± 0.67	29.14** ± 2.7	9.25 ± 0.6	25.3** ± 1.3	F=1.60	F=99.11	F=0.74
		193%		174%	NS	P<0.01	NS

Experimental details are given in method's section. All values are mean ± SEM of five rats in each group (n=5). Treated groups were administered various doses of fluoxetine dissolved in vehicle (DMF:Saline, 1:3 v/v). Control groups received an equal volume of vehicle at dose of 2ml/kg. Statistical analysis was performed using TWO-WAY ANOVA followed by Newman-Keuls test. The significance of difference is indicated by * P<0.05 and ** P<0.01 from respective controls. NS indicates the non-significant effects.

CONCLUSION

Despite of the higher activity of holo-enzyme in females the extent of inhibition of tryptophan pyrrolase activity by fluoxetine is similar but the better response shown to SSRIs' treatment in depression by women could be due to high-storage capacity and slower hepatic metabolism thus prolonging the stay of the drug in the body, however this needs further investigation. Conversely, the higher vulnerability to depression could be attributed to higher activity of the holo-enzyme in females. More detailed investigations on tryptophan metabolism and disposition after drug administration in both sexes will be fruitful to reach at comprehensive conclusion.

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