INTRODUCTION

Hormonal Contraceptive (HC) have three combination types, one having a fixed dose of a combination of synthetic estrogen and a synthetic progestin, the second has a varying doses of each of these steroids and the third being a fixed dose of a progestin without an estrogen.\(^1\) The main action of combined administration of progestin and estrogen involves the inhibition of ovulation by an interference with hypothalamic pituitary mechanism.\(^2\)

Apart from inhibition of pregnancy, there are a variety of major and minor side effects attributed to the use of HC, such as depression, alteration in lipid profile, cardiovascular disorders, breast cancer, hypertension, vitamin B-6 deficiency and glucose intolerance.\(^1\) Depression associated with oral contraceptive agent may differ significantly from organic major depressive episode.\(^1\) Drug-induced depression is classified by DSM-R (Diagnostic Statistical Manual, Revised) as an organic mood syndrome of the depressed type.\(^3\)

Study on the effect of the administration of a combined estrogen and progesterone contraceptive agent on tryptophan metabolism has shown increased urinary excretion of metabolite of tryptophan and nicotinic acid after a loading dose of tryptophan whereas progesterone alone caused a decrease in urinary excretion of nicotinic acid metabolites.\(^4\) Depressive illness may complicate the use of HC (hormonal contraceptives), a result of functional deficiency of pyridoxine, with impaired activity of enzyme tryptophan pyrrolase, the first and rate limiting enzyme of tryptophan metabolism. This reaction is via kynurenine-nicotinamide pathway and takes place in the liver where tryptophan is fed into relevant metabolic pathways.\(^5\)

Oral contraceptive seems to affect on mood and behaviors in some women with pre-existing psychiatric illness, sometime inducing depression.\(^6\) Changes induced by contraceptive steroids on tryptophan metabolism and corticosteroid metabolisms were correlate with the associated depression in susceptible women.\(^7\) Depressive mood has been linked to the activity of serotonin or 5HT and norepinephrine in the brain. 5HT and norepinephrine are neurotransmitters that effect mood, a higher level of 5HT and norepinephrine activity is associated with positive mood while decreased activity is.

ORIGINAL ARTICLE

INHIBITION OF TRYPTOPHAN PYRROLASE ACTIVITY IN RESTRAINT FEMALE RATS FOLLOWING MEDROXYPROGESTERONE ADMINISTRATION

Shabana Saeed and Samina Bano

ABSTRACT

Objective: To determine the effects of Medroxyprogesterone (hormonal contraceptive) in restraint stressed female rats in relation to tryptophan metabolism.

Design: Pre-clinical study.

Place and Duration of Study: Department of Biochemistry, University of Karachi. The investigation was carried out in the year 2003.

Materials and Methods: Female Albino Wistar rats (150-200 gm body wt) were selected and divided into four groups (n=5 in each group). Rats were injected intraperitoneally either vehicle or Medroxyprogesterone (25mg/kg/ml) and were immediately subjected to 2 hours restraint stress while respective controls remained in their home cages.

Results: In restraint stress group, hepatic holo and total tryptophan pyrrolase activities were increased. Liver tryptophan, total serum tryptophan and albumin concentration were decreased. Brain tryptophan, 5HT and 5HIAA concentrations were increased. Medroxyprogesterone administration in unrestraint rats inhibited holo, total and apo enzyme activities with increases in liver tryptophan concentrations. Effect of restraint stress following Medroxyprogesterone administration when compared with drug injected unrestrained group showed increase in holo and total tryptophan pyrrolase activities with decrease in liver tryptophan concentrations. Brain tryptophan, 5HT and 5HIAA levels were increased. Results when compared with vehicle injected stressed-rats showed that total and apo tryptophan pyrrolase activities were decreased. Liver tryptophan, serum tryptophan and albumin concentrations were increased but brain tryptophan metabolism was not effected.

Conclusion: It is concluded that Medroxyprogesterone inhibits stress induce increases in peripheral tryptophan metabolism and increases plasma tryptophan. Although stress induced increases in brain indoles were not effected by the drug at two hours, further studies on time course effects of this drug will be needed to explore its possible anxiolytic effects.

KEY WORDS: Medroxyprogesterone. Stress. Tryptophan. 5-HT. 5-HIAA.
associated with depressive mood. Estrogen can augment both reduced serotonergic activity and some norepinephrine related processes. Treatment with estrogen alone may improve mood by boosting the activity of serotonin in women with mild depressive symptoms but moderate to severe depression need antidepressant therapy, while progesterone can reduce anxiety and related symptoms. Combination of stress and alteration in sex hormones may be responsible for mood changes during pill-free period in women taking HC. Rats exposed to a single 2 hours restraint stress exhibit reduced locomotion and increased defecation in an open field (behavioral test) 24 hours later, together with a marked anorexia and decreased growth rate. Such stress-induced behavioral deficits have been widely used as animal model of depression.

The objective of the present study was to investigate changes in tryptophan metabolic pathway in female rats after 2 hours restraint stress following hormonal contraceptive (Medroxyprogesterone) administration.

**Materials and Methods**

Locally bred female rats weighing (200-250 g) were used for the experiment. The rats were housed in plastic cages in a quiet room with free access to cubes of standard rat food and water for at least 4-5 days before starting the experiments so that the rats could adapt themselves to the new environment. Injectable hormonal contraceptive Megesterone 150 mg/ml (Medroxyprogesterone) manufactured by N.V.Organon Oss (Holland) was used. All chemical used were purchased from a single source.

Rats were divided in to four groups. Each group had five rats. 1st and 2nd groups were injected with drug in concentration of 25 mg/kg/ml, dissolved in ethanol: saline (1:6). The 1st group was immediately immobilized by being taped to metal grid for 2 hours, but 2nd group remained in their home cages. The 3rd and 4th groups received the vehicle (ethanol: saline) only. The 3rd group was immediately immobilized but the 4th group remained in their home cages for 2 hours. Animals were killed by decapitation after 2 hours. Perfused liver, plasma and brain were isolated and stored at −70°C until analyzed. Livers were perfused in situ via hepatic portal vein with ice cold 0.95% NaCl to flush out the blood. Liver tryptophan pyrrolase activity was determined in homogenates either in the absence (holo enzyme activity) or in the presence (total enzyme activity) of added he (2µm) hematin as described in detail by earlier researchers. The apo enzyme activity was obtained by difference (total enzyme activity– holo enzyme activity). Other parameters of tryptophan metabolism such as liver and serum tryptophan concentrations and brain tryptophan, 5-HT and 5-HIAA concentrations were determined by established procedures. Serum albumin concentrations were determined by standard clinical procedure.

The data was analyzed by two-way ANOVA [Factor 1= restraint stress, Factor 2 = drug and interaction (drug x restraint) between the two factors]. Individual comparison was made by using Newman-Keuls-Q Statistics. Differences between groups were considered significant when \( p<0.05 \).

**Results**

Table I shows the effects of 2 hours restraint stress on tryptophan pyrrolase activity in vehicle and Medroxyprogesterone injected female rats. Data analyzed by two–way ANOVA shows effects of restraint, drug and restraint x drug interaction on tryptophan pyrrolase activities. Effects of restraint were significantly higher on holo enzyme and total enzyme activities \( F=37.2 \) (p<0.01) and \( F=15.1 \) (p<0.01) respectively. Effect of drug were significant on total and apo enzyme activities \( F=28.1 \) (p<0.01) and \( F=20.2 \) (p<0.01).

**Table I** Effect of restraint on tryptophan pyrrolase activity in medroxyprogesterone (25 mg/kg/ml) injected female rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Tryptophan pyrrolase activity # moles of kynurenine formed/h/g/wet wt of liver</th>
<th>TWO-WAY ANOVA</th>
<th>DF 1,16</th>
<th>Restraint X drug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unrestraint DRUG Unrestraint DRUG Unrestraint DRUG Unrestraint DRUG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOLO Enzyme</td>
<td>1.44±0.15 2.33±0.3* 0.97±0.03†† 2.36±0.06** F= 37.18 P&lt;0.01 F=3.0 N.S F= 3.90 N.S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change from respective control</td>
<td>+ 61.8% - - - -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change from similarly treated saline injected rats</td>
<td>- - +143.3% - - -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL Enzyme</td>
<td>3.56±0.4 4.42±0.15* 2.15±0.16†† 5.22±0.12**†† F=15.12 P&lt;0.01 F=28.10 P&lt;0.01 F=0.13 N.S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change from respective control</td>
<td>+30.8% - - +49.7% - -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change from similarly treated saline injected rats</td>
<td>- - 39.6% -30.8%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apo Enzyme</td>
<td>2.12±0.24 2.11±0.31 1.17±0.14† 0.67±0.07†† F=0.48 N.S F=20.2 P&lt;0.01 F=1.82 N.S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change from respective control</td>
<td>- 0.004% - - +42.7% - -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change from similarly treated saline injected rats</td>
<td>- - 44.8% -68.2%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All values are mean ± SEM (n=5). Test groups administered Medroxyprogesterone (25 mg/kg/ml) dissolved in vehicle (Ethanol: Saline: 1: 6 v/v). Control groups received an equal volume of vehicle. Statistical analysis performed using two-way ANOVA followed by Newman-Keuls Q test. The significance of difference is indicated by \(*P<0.05\) and \(**P<0.01\) from respective control. \(\dagger P<0.05\) and \(\dagger\dagger P<0.01\) from similarly treated vehicle injected female rats N.S. indicates the non-significant difference.
respectively. The interaction between the two (restraint x drug) was not significant. Individual comparison by Newman-Keuls-Q Statistics showed significant induction by 61.8% (p<0.05) in holo and 30.8% (p<0.05) in total enzyme activities while apo enzyme activity was not effected in restraint stress when compared with respective controls. Effect of drug in un-restraint rats showed that holo, total and apo enzyme activities were significantly inhibited by 32.6% (p<0.01), 39.6% (p<0.01) and 44.8% (p<0.05) respectively when compared with similarly treated vehicle-injected rats. The effect of drug in restraint rats showed significant induction by 143.3% (p<0.01) and 49.7% (p<0.01) respectively in holo and total enzyme activities while apo enzyme activity was not effected when compared with respective controls. The effect of drug in restraint rats showed that total and apo enzyme activities were significantly inhibited by 30.8% (p<0.01) and 68.2% (p<0.01) respectively when compared with similarly treated vehicle injected rats.

Figure 1 shows individual comparison by Newman-Keuls Q-test on liver tryptophan concentration in unrestraint and restraint rats. Effect of restraint on saline injected rats showed significant decrease by 43.8% (p<0.01) when compared with respective controls (unrestraint). Effect of drug in unrestraint rats showed significant increase by 35.9% (p<0.01) when compared with similarly treated saline-injected rats. There was a significant increased by (53.5% p<0.01) in restraint drug injected rats when compared with similarly treated saline-injected rats. The effect of restraint in drug injected rats showed significant decreases by 36.5% (p<0.01) when compared with respective control (unrestraint). Table II shows the effect of 2 hours restraint stress in saline injected rats and Medroxyprogesterone injected female rats on serum total tryptophan and albumin concentrations. Data analyzed by two-way ANOVA showed significant effects of restraint on serum tryptophan and albumin concentrations F=8.58 (p<0.01) and F=9.25(p<0.01) respectively. The effects of drug on serum tryptophan and albumin concentrations F=4.71(p<0.05) and F=35.63(p<0.01) were also significant. Interaction between restraint x drug was significant only on albumin concentration F=22.3 (p<0.01).

Individual comparison by Newman-Keuls Q-Statistics showed that there was significant decreased by 25.9% (p<0.05) due to restraint stress on serum tryptophan and 34.7% (p<0.01) on albumin concentrations. Effects of drug in unrestraint rats were not significant on serum tryptophan and albumin concentrations when compared with vehicle-injected rats. Effects of restraint in drug injected rats showed that there was significant increased in serum tryptophan and albumin concentrations by 53.5% (p<0.01).
concentrations by 29.4% (p<0.05) and 73.4% (p<0.01) respectively when compared with similarly treated saline injected rats. There were no effects of restraint following Medroxyprogesterone when compared with respective control.

Table III shows effect of 2 hours restraint on brain indoles in saline and Medroxyprogesterone injected female rats. Data analyzed by two-way ANOVA showed effect of restraint, drug and restraint x drug on brain indole concentrations. The effects of restraint were significant on brain tryptophan, 5-HT and 5-HIAA concentrations F=25.19 (p<0.01), F=21.03 (p<0.01) and F=13.72 (p<0.01) respectively. Effects of drug and the interaction between two (restraint x drug) were not significant on brain tryptophan, 5-HT and 5-HIAA.

Individual comparison by Newman-Keuls Q Statistics showed that there were significant increase by 32.8% (p<0.05) in brain tryptophan, 37.5% (p<0.05) in 5-HT and by 81.81% (p<0.01) in 5-HIAA concentrations due to restraint stress. Effects of drug in unrestraint rats were not significant in brain tryptophan, 5-HT and 5-HIAA concentrations when compared with similarly treated vehicle injected rats. Effect of restraint in drug injected rats showed significant increase by 56.4% (p<0.01) in brain tryptophan, by 56.3% (p<0.01) in 5-HT and 64.1% (p<0.05) in 5HIAA concentrations when compared with control (unrestraint drug injected rats). There was no effect of drug on brain indoles concentrations in restraint rats when compared with similarly treated vehicle injected rats.

**DISCUSSION**

The present study shows that a 2 hours restraint stress in female rats increases tryptophan pyrrolase activity. Also similar increases have been reported earlier suggesting that stress activates not only serotonergic pathway but also kynurenine pathway in the peripheral and central nervous system. It was also suggested that stress also elevates the level of kynurenine in the plasma, liver, kidney and every part of the brain. Studies have shown that stress induced increases in tryptophan pyrrolase is mediated by the action of glucocorticoids. Induction of glucocorticoids receptors in rat liver has been reported earlier in rats subjected to various stressful stimuli e.g. burns, tumors and partial heptectomy. In the present study, changes in enzyme activities are not reflecting to decrease in liver tryptophan concentration but decreases in serum total tryptophan levels. Such decreases has also been reported in rats after immobilization or food deprivation rats leading to substantial increase in brain tryptophan. Moreover, it has also been reported that immobilization increases brain tryptophan concentration increasing free tryptophan in plasma. Free tryptophan was not determined in present study but it is more likely that free tryptophan concentration would have increased. In the present studies, restraint stress increases brain 5-HT turnover by increasing the availability of tryptophan to the brain (Table III), such increases has also been shown previously. There is a significant increase in brain tryptophan, 5-HT and 5-HIAA concentrations.

Stress induced increase free tryptophan and thus 5-HT is mediated by norepinephrine. It is possible that the norepinephrine released acts as a governor or restraint for the feed-forward effects of the serotonin system. Synthesis of tryptophan is of interest in stress. The AA (amino acid) is carried in the blood stream primarily bound to albumin at the FA (fatty acid) side. The uptake into the brain is a result of a

**Table III**: Effect of 2 hours restraint on brain indoles in saline and medroxyprogesterone (25 mg/kg/ml) injected female rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>VEHICLE</th>
<th>DRUG</th>
<th>TWO-WAY ANOVA</th>
<th>DF 1, 16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unrestraint</td>
<td>Restraint</td>
<td>Unrestraint</td>
<td>Restraint</td>
</tr>
<tr>
<td>Brain Tryptophan</td>
<td>1.46 ± 0.14</td>
<td>1.94 ± 0.13*</td>
<td>1.17 ± 0.08</td>
<td>1.83 ± 0.07**</td>
</tr>
<tr>
<td>% change from respective control</td>
<td>+32.8%</td>
<td>+56.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change from similarly treated saline injected rats</td>
<td>-19.86%</td>
<td>-5.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain 5-HT</td>
<td>0.56 ± 0.01</td>
<td>0.77 ± 0.06*</td>
<td>0.55 ± 0.05</td>
<td>0.86 ± 0.06**</td>
</tr>
<tr>
<td>% change from respective control</td>
<td>+37.5%</td>
<td>+56.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change from similarly treated saline injected rats</td>
<td>-1.7%</td>
<td>+11.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain 5-HIAA</td>
<td>0.44 ± 0.04</td>
<td>0.80 ± 0.04**</td>
<td>0.39 ± 0.05</td>
<td>0.64 ± 0.06*</td>
</tr>
<tr>
<td>% change from respective control</td>
<td>+81.81%</td>
<td>+64.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change from similarly treated saline injected rats</td>
<td>-11.36%</td>
<td>-20%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All values are mean ± SEM (n=5). Test groups were administered Medroxyprogesterone (25 mg/kg/ml) dissolved in vehicle (Ethanol: Saline, 1:6 v/v). Control groups received an equal volume of vehicle. Statistical analysis was performed using two-way ANOVA followed by Neuman-Keuls Q test. The significance of difference is indicated by *P<0.05 and **P<0.01 from respective control. N.S indicates the non-significant difference.
facilitated transport system. The free concentration of tryptophan is increased by FFA (free fatty acid) in the blood. Epinephrine and cortisol increase FFA. This increase in availability of tryptophan acts to increase serotonin synthesis.\textsuperscript{24} This suggests a common pathway for the central transduction of stress onto the adrenal cortex and adrenal medulla.\textsuperscript{25}

It was found that in absence stress Medroxyprogesterone decreases holo, total, apo tryptophan pyrrolase activity and increases liver tryptophan concentrations. Estrogen, progesterone and pregnancy all inhibit liver tryptophan pyrrolase activity thus increase tryptophan availability to brain in rat or man.\textsuperscript{26}

It was reported that progesterone mainly inhibits pyrrolase activity by prevention of conjugation of apo-enzyme with haem and decreased haem availability. The hormone may be involved in the tryptophan pyrrolase inhibitory effects during pregnancy and in oral contraceptive users.\textsuperscript{27} The hepatic cytosolic hemoprotein tryptophan pyrrolase is the rate-limiting enzyme in the tryptophan catabolism and thus plays a key role in regulating flux of tryptophan into relevant metabolic pathways. The tryptophan pyrrolase protein is stabilized by its prosthetic haem. Hepatic haem deficiency may enhance the tryptophan flux into synthetic (serotonergic) pathways, not only by depriving prosthetic haem for functional competent tryptophan pyrrolase hemoprotein, its primary catabolic enzyme, but also by impairing the denovo synthesis of this enzyme.\textsuperscript{28} After progesterone treatment, the changes in peripheral tryptophan metabolism were not reflected in cerebral changes in brain tryptophan, 5-HT and 5-HIAA concentrations.

Although studies\textsuperscript{29} have shown decreases in brain tryptophan, 5-HT and 5-HIAA, after chronic administration of Medroxyprogesterone, decreases in brain indoles were not effected by the drug at two hours, further studies on time course effects of this drug will be needed to explore its possible anxiolytic effects.

Present study gives an insight to HC effects on mood in relation to its effects on brain 5-HT and its use in women suffering from mood disorder such as anxiety and depression.

**Conclusion**

It is concluded that Medroxyprogesterone inhibits stress induce increases in peripheral tryptophan metabolism and increases plasma tryptophan. Although stress induced increases in brain indoles were not effected by the drug at two hours, further studies on time course effects of this drug will be needed to explore its possible anxiolytic effects.

**Acknowledgement:** The author thank the office of Dean, Faculty of Science, and University of Karachi for the financial support for the project.

**References**


