ACUTE EFFECTS OF SEROTONERGIC ANTIDEPRESSANTS ON TRYPTOPHAN METABOLISM AND CORTICOSTERONE LEVELS IN RATS

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ABSTRACT

The aim of present study is to see the effects of antidepressants in relation to tryptophan metabolism and disposition and to know whether they share any common mechanism of action in this regard. These are the monoamine oxidase inhibitor (moclobemide), atypical tricyclic (tianeptine), selective serotonin reuptake inhibitors (SSRIs) namely sertraline and citalopram and an herbal St John's Wort (SJW). Liver tryprophan pyrrolase activity, serum tryptophan, corticosterone and brain indoles were determined after drug administration in Albino Wistar rats at a dose of 10mg/kg. All five antidepressants inhibited tryptophan pyrrolase activity. Serum total tryptophan concentrations were increased by 19% and 33% by tianeptine and moclobemide respectively, however 34% decrease in total tryptophan was observed after SJW administration. Free tryptophan was increased by all the drugs being maximum (65% $P<0.001$) by sertraline and minimum (15%, $P<0.05$) by tianeptine. Corticosterone levels were significantly ($P<0.01$) decreased by 52 and 58 percent by citalopram and St John's Wort respectively. By contrast an increase by 16% was observed by tianeptine. It was also observed that all the drugs increase brain tryptophan by 21-61 percent but increases in 5-hydroxytryptamine (5-HT) were observed only by two drugs that is moclobemide and SJW, however in comparison increases were greater (68%) after SJW administration. 5-hydroxyindoleacetic acid (5HIAA) concentrations were increased by 45-64% by all other drugs except tianeptine and moclobemide. It is concluded that attenuation of peripheral tryptophan metabolism and elevation of brain tryptophan contributes to the mechanism of action of antidepressants of different classes and pharmacological profile tested.

Keywords: Antidepressants, corticosterone, 5-HT, tryptophan, tryptophan pyrrolase.

INTRODUCTION

It was shown previously that 23 antidepressants increases rat brain tryptophan and thereby enhances 5-HT synthesis by inhibiting liver tryptophan pyrrolase activity (Badawy & Evan 1981; Badawy & Evans 1982; Badawy & Morgan 1991).In present work we have examined 5 more antidepressants in current clinical use on tryptophan metabolism and disposition. Keeping in view evidences presented earlier it was desired to know the acute effects of 10mg/kg doses of the monoamine oxidase inhibitor (moclobemide), atypical tricyclic (tianeptine), selective serotonin reuptake inhibitors (SSRIs) namely sertraline and citalopram and St john's Wort (SJW) on tryptophan metabolism and disposition and also to know whether they share any common mechanism of action or not. SJW is the only herbal antidepressant considered to be an effective alternative to other therapeutic agents in the treatment of mild to moderate depression. Several constituents of the SJW extracts such as hypericum and hyperforin are important for this effect. The mechanism of action of is still under investigation but like other conventional antidepressants, it has also been demonstrated for the inhibition of neural uptake of serotonin, norepinephrine and dopamine (Mennini & Gobbi 2004).

The monoamine hypothesis of depression implies that the low levels of brain 5HT and noradrenaline leads to depressive symptoms (Van Pragg, 1978; Wong & Licinio, 2001). The disturbance of serotonergic system may be one of the essential factors implicated in the etiology of mental disorders including such complex pathologies like schizophrenia and depression (Hardina *et al*., 1993; Joyce, 1993; Maes & Meltzer 1995).The serotonin neuro-transmitter system has been extensively investigated in relation to the biology of depression and the mechanism of antidepressant action. Evidence for the involvement of serotonin in depression comes from elevated serotonergic function with chronic administration of various types of antidepressant treatments (Blier *et al*., 1988). Antidepressants drugs may act in part by enhancing serotonergic activity. The serotonergic deficit may occur at any of several levels: diminished availability of precursor, impaired activity of tryptophan hydroxylase, abnormalities in 5-HT release or uptake, 5-HT receptor abnormalities or interaction with other neurotransmitters. The synthesis of serotonin in the neuronal cells is

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regulated by a number of factors including substrate or precursor availability and end product inhibition by its metabolites. The substrate availability is affected by amount of L-tryptophan in diet and activity of the liver cytosolic enzyme tryptophan pyrrolase (tryptophan-2, 3-dioxygenase; EC1.13.11.11). This by quantitative estimations is the most important enzyme involving 99.00% degradation of the amino acid via kynurenine-nicotinamide pathway (Badawy, 1977). This enzyme is induced by the stress hormone cortisol and tryptophan itself. The hyperactivity of the pituitary-hypothalamic adrenal axis results in enhanced release of cortisol (Nemeroff, 1998) that causes induction of tryptophan pyrrolase resulting in increased degradation of the L-tryptophan and this spares little amino acid to be taken up by the neuronal cells. The importance of tryptophan pyrrolase in regulation of serotonin synthesis is evident from the observation that majority of patients suffering from depression have elevated cortisol levels (Salter & Pogson, 1985; Curzon, 1988). One sequel of prolonged cortisol increase is a reduction in serotonin synthesis. Excessive cortisol activates the enzyme tryptophan pyrrolase, shunting the dietary amino acid precursor tryptophan away from the serotonin pathway and into the kynurenine to niacin pathway resulting in inhibited serotonin production and reduced sensitivity of serotonin receptors (Linde *et al*., 1996).

However a problem that remains unsolved by the introduction of the selective serotonin reuptake inhibitors (SSRIs) and which is also shared by other antidepressants is a slow onset of antidepressants action of approximately 2-3 weeks (Hollister & Claghorn, 1993). A rapid attainment of high synaptic 5-HT may enhance the onset of postsynaptic changes in emotive processing and hasten the therapeutic on set. This hypothesis is supported by clinical data which shows a faster on set of antidepressant action with combination of reuptake inhibitor and 5-HT precursor tryptophan (Walinder *et al*., 1976).

The efficacy of tryptophan as an antidepressant alone or in combination is limited because of its rapid metabolism by the major site of tryptophan catabolism in the body, the kynurenine pathway in the liver (Moller *et al*., 1982). Efficacy may also be limited because of intra-neuronal metabolism of newly synthesized 5-HT by monoamine oxidase (MAO) (Sharp *et al*., 1992). Inhibitors of this enzyme should by inhibiting the catabolism of endogenous tryptophan in the body, elevate plasma tryptophan (and therefore brain tryptophan). This will in turn increase the saturation of tryptophan hydroxylase which is known to be 50% saturated with tryptophan under normal conditions. 5-HT levels will be more sensitive to decrease in tryptophan than increase in tryptophan availability in the brain (Pogson *et al*., 1989).

Present results helps us to gain insight into the mechanism of action of drugs in relation to the possible involvement of disturbed hepatic tryptophan metabolism and thus 5-HT in depressive illness and their effect on hypothalamic pituitary adrenal (HPA) axis as it may be an important target for antidepressants induced clinical improvement in patients diagnosed with depression and who demonstrate hyper-cortisolaemia.

MATERIAL AND METHOD

Animals and Treatment

Locally bred adult male Albino Wistar rats (150- 200g body wt.) were housed five rats per cage under natural light dark cycle at constant temp 22°C±2°C room temperature relative humidity of 60% and were maintained on lab chow and water *ad libitum.* Rats were divided into 9 groups. Each group had five rats. Test groups received intraperitoneal (i.p) administration of antidepressants 10m/kg each and respective control groups received an equal volume of vehicle (DMF: saline 1:3 v/v). Animals were killed by decapitation after two hrs for tryptophan pyrrolase activity. Serum parameters and brain indoles were measured at 3.5hrs after drug administration. Perfused livers (perfusion *in situ* with ice cold saline) and brains samples taken out were stored at -20°C until analysis. Serum was also isolated; a 1ml portion of each fresh serum was also ultra filtrated using micro-partition assembly (for determination of free tryptophan) and was frozen along with the parent serum. Ethical approval was obtained from Cardiff School of Medicine Research Ethics Committee.

Enzymatic and other Determination

Tryptophan pyrrolase activity was determined in homogenates prepared from frozen livers using ultra-turrax homogenizer either in the absence (holo enzyme activity) or in the presence (total enzyme activity) of added (2μM) haematin (dissolved in 0.1M NaOH) as described earlier (Bano & Sherkheli, 2003) . The apo-enzyme activity was obtained by difference of total enzyme activity and holo enzyme activity (Badawy & Evans 1976). Brain tryptophan, 5-HT and its major metabolite 5-HIAA were determined spectro-fluorimetrically (Bano *et al*., 1996). Serum and liver tryptophan concentrations, were determined by modification (Bloxam & Warren, 1974) of the fluorimetric method of Denckla and Dewey, 1976. Serum corticosterone was measured by Glick *et al*. (1964).

Drugs and chemicals

Haematin hydrochloride and L-tryptophan from Sigma chemical Co (St, Louis, Mo); Antidepressants used were pure, gift from pharmaceuticals, these are sertraline (Pfizer, UK), citalopram hydrobromide (Lundbeck, UK), Moclobemide (Roche, UK), tianeptine sodium (Servier,

France) and Saint John's Wort (Medics Pharma, Pakistan). All others chemicals used were of highest analytical grade.

Statistical analysis

Data are expressed as mean ± standard error of mean. Analysis was performed by using student's *t*- test. Differences between the two groups were considered significant when P<0.05.

RESULTS

Effects of administration of antidepressants on the basal activity of rat liver tryptophan pyrrolase activity

Table 1 shows that 2h after administration of the tianeptine the inhibition of total enzyme and apoenzyme activities was 40% (P<0.001) and 67% (P<0.001) respectively. Administration of citalopram inhibited the total enzyme activity by 32% (P<0.01) and apoenzyme activities by 73% (P<0.001). Administration of moclobemide and sertraline inhibited total enzyme activities by 31% and 41% while apoenzyme was inhibited by 55% and 80% respectively. In contrast to other drugs herbal St. John's Wort (SJW) inhibited holoenzyme activity by 24%. Inhibition of total enzyme was only 10% but was significant while there was no effect on apo enzyme activity.

Effects of antidepressants on liver and serum tryptophan concentration

Table 2 shows that total serum tryptophan concentrations were increased by tianeptine and moclobemide by 19% and 33% respectively in contrast serum tryptophan concentrations were decreased by 34% by SJW. Serum free tryptophan was increased by all drugs by 15%-65% being minimum by tianeptine and maximum by sertraline. Liver tryptophan concentrations were increased by all antidepressants except moclobemide.

Effects of antidepressants on rat brain indoles

Table 3 shows that brain tryptophan concentrations were increased by 21-61 percent by all antidepressants with

Table 1: Effects of antidepressants on the basal activity of rat liver tryptophan pyrrolase activity

Treatment	Tryptophan pyrrolase activity(μ mol of kynurenine formed/h/g/wet.wt of liver)		
	Holo enzyme activity	Total enzyme activity	Apo enzyme activity
Saline	2.5±0.26	4.5±0.25	2.0±0.05
Moclobeamide	2.2±0.17	3.1±0.12**	0.9±0.24*
Sertraline	2.3±0.09	2.7±0.00**	0.4±0.09**
Saline	1.6±0.16	3.1±0.2	1.5±0.20
Citalopram	1.7±0.07	2.1±0.05*	0.4±0.08**
Saline	1.7±0.03	3.8±0.12	2.1±0.11
Tianeptine	1.6±0.16	2.3±0.20**	0.7±0.05**
Saline	2.1±0.1	4.1±0.06	2.1±0.07
St.John's Wort	1.6±0.12*	3.7±0.04**	2.0±0.08

Experimental details are as described in the materials and methods section. Measurements were performed at 2h after i.p. administration of drugs 10mg/kg each or an equal volume 2ml/kg of the vehicle (DMF: Saline 1:3 v/v). Values are means± SEM for each group of 5 rats per experiment. The effects of drugs are compared with the results in saline-treated controls. The significance of the differences is indicated as follows:*P<0.01, **P<0.001.

Table 2: Effects of antidepressants on liver and serum tryptophan concentration

Treatment	Serum tryptophan μg/ml	Liver tryptophan μg/g	Serum free tryptophan μg/g	% Free tryptophan
Saline	19.63±0.58	5.44±0.22	1.06±0.05	5.4±0.25
Sertraline	20.38±0.71	6.22±0.26*	1.75± .07***	8.59±0.33**
Citalopram	20.60±1.24	6.69±0.17**	1.62 ±0.07***	7.86 ± 0.66**
Saline	17.08±0.32	3.70±0.14	0.98±0.06	5.74± 0.34
Tianeptine	20.26±0.75**	4.07±0.13*	1.13±0.08*	5.58 ± 0.43
Moclobemide	22.66±0.50***	4.01±0.26	1.17±0.08*	5.16 ± 0.38
Saline	15.11±1.65	7.8±0.3	0.84±0.02	5.56 ± 0.13
St.John's Wort	10.0±0.27**	9.0±0.01**	1.06±0.05**	10.60±0.50**

Experimental details are as described in the materials and methods section. Measurements were performed at 2h after i.p administration of drugs 10mg/kg each or an equal volume 2ml/kg of the vehicle (DMF: Saline 1:3 v/v). Values are means± SEM for each group of 5 rats. The significance of the differences is indicated by *P<0.05, **P<0.01, ***P<0.001.

maximum increase by tianeptine. Increases in 5-HT concentrations were observed by moclobemide and SJW only however the increases were greater by the later drug. 5-HIAA concentrations were decreased by sertraline and citalopram by 45% (P<0.001) and 49% (P<0.001) respectively. In contrast there was increase by 64% (P<0.001) in 5-HIAA concentrations by SJW.

Effects of antidepressants, on rat serum corticosterone concentrations

Fig. 1 shows serum corticosterone levels were not affected by sertraline but there were significant decreases by 52 and 58 percent by citalopram and St John's Wort respectively, by contrast an increase by 16% was observed by tianeptine, however increases after moclobemide administration were not significant.

Effects of antidepressants on rat serum corticosterone Concentrations

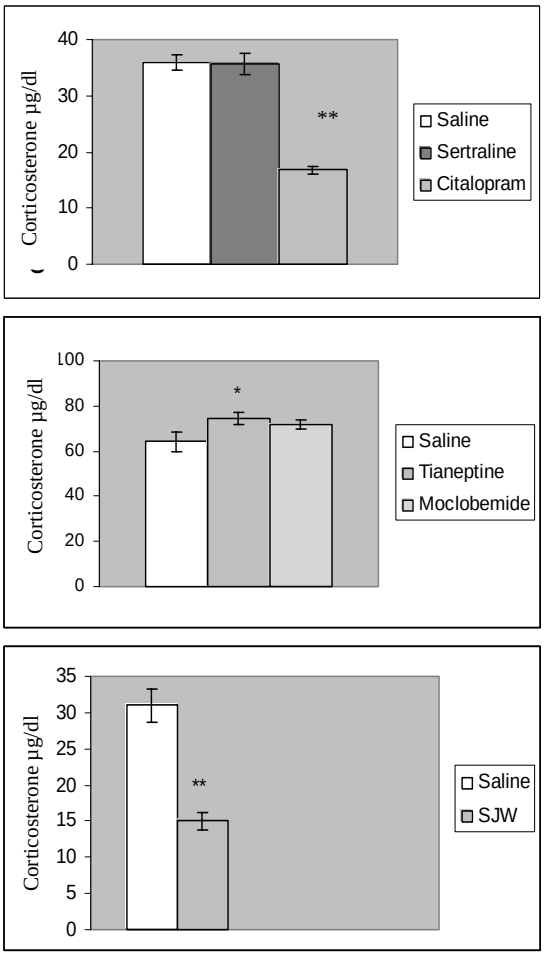

Fig. 1: Experimental details are as described in the materials and methods section. Measurements were performed at 3.5h after i.p. administration of drugs 10mg/kg each or an equal volume 2ml/kg of the vehicle (DMF: Saline 1:3 v/v) values are means± SEM for each group of 5 rats. The significance of the differences is indicated by * P<0.05, **P<0.001.

DISCUSSION

Cerebral 5-hydroxytryptamine (serotonin, 5-HT) synthesis is controlled mainly by brain tryptophan concentrations, because the rate limiting enzyme of the 5-HT biosynthetic pathway, tryptophan hydroxylase, is unsaturated with its substrate (tryptophan). This process is determined by at least three peripheral factors influencing circulating tryptophan availability to the brain that play important roles in central 5-HT synthesis. These factors include tryptophan binding to circulating albumin, competition between tryptophan and large neutral amino acids (LNAA), namely valine, leucine, isoleucine, phenylalanine & tyrosine for cerebral uptake (Fernstrom & Wurtman, 1971) and activity of the major tryptophan degrading enzyme, liver tryptophan pyrrolase activity.

The present study demonstrates the ability of 5 antidepressants to inhibit tryptophan pyrrolase activity after administration. The inhibition of apoenzyme by all antidepressants except St John's Wort (SJW) appears to be caused by an interference with the conjugation of apoenzyme with its cofactor haem. It remains to establish whether it is due to defective apoenzyme synthesis or lowering of corticosterone concentrations as suggested by Samsonova and lapin (1973). Earlier it has been reported (Badawy & Evans, 1981; Badawy & Evans, 1982) that antidepressants differ from ethanol, glucose or nicotinamide whose chronic administration inhibits pyrrolase activity by increasing the hepatic concentrations of allosteric inhibitor; nicotinamide adenine dinucleotide phosphate (reduced). The ability of administered anti-depressants to inhibit pyrrolase activity may depend on the extent of the haem saturation of the apoenzyme and therefore on the status of liver haem. We have not examined the effects of antidepressants on the cortisol induction or the tryptophan activation of tryptophan pyrrolase. However, in view of the similarity of action of antidepressants with exception of herbal SJW, it may be suggested that antidepressants may completely block the tryptophan activation of pyrrolase whereas they may only prevent the cortisol induced increase in the apoenzyme but not in the holoenzyme activity.

It is well established that hypothalamic pituitary adrenal axis dysregulation characterized by elevated circulating cortisol concentrations and impaired negative feed back inhibition is associated with affective disorders. As normalization of the HPA axis function and mood stabilizing effects occur simultaneously during antidepressant treatment it is likely that these effects are either directly or indirectly dependent. It is thought that antidepressants may normalize the HPA axis hyper-drive via re-establishment of the feedback control (Maes & Meltzer, 1995; Boyle *et al*., 2005). In present study Tianeptine administration have been found to increase plasma corticosterone levels slightly but significantly

Table 3: Effects of antidepressants on rat brain indoles.

	Brain indoles concentration (μ g/g wet wt)		
Treatment	Tryptophan	5-HT	5-HIAA
Saline	1.24±0.11	0.64±0.07	0.29±0.02
Sertraline	1.94±0.12**	0.60±0.01	0.16±0.01***
Citalopram	1.99±0.09**	0.64±0.02	0.15±0.016***
Saline	1.20±0.05	0.50±0.03	0.34±0.00
Tianeptine	1.67±0.07***	0.54±0.02	0.33±0.01
Moclobemide	1.6±0.10**	0.6±0.03*	0.32±0.01
Saline	1.2±0.10	0.6±0.04	0.33±0.03
St.John's Wort	1.45±0.04*	1.01±1.04*	0.54±0.02***

Experimental details are as described in the materials and methods section. Measurements were performed at 3.5h after i.p administration of drugs 10mg/kg each or an equal volume 2ml/kg of the vehicle (DMF: Saline 1:3 v/v). Values are means± SEM for each group of 5 rats. The significance of the differences is indicated by*P<0.05, **P<0.01, ***P<0.001.

however Delbende & Coworkers (1991) have found that single injection of tianeptine (10mg/kg) significantly decreased corticosterone levels in rats subjected to restraint stress but in contrast Broqua *et al*. (1992) have reported that similar doses have no effect on restraint induced increase in plasma corticosterone levels. Later other investigators (Mennini *et al*., 1993) also reported that tianeptine at the dose of 10mg/kg 1h before stress did not prevent rise in plasma corticosterone levels in stressed rats. We have also found citalopram decreases corticosterone levels at 2h after intraperitoneal administration in contrast earlier Jensen *et al*. (1999) had reported that citalopram (10mg/kg) injected subcutaneously increases corticosterone levels. This difference in effects of citalopram could be due to difference in the route of administration. Herbal SJW in present study decreases corticosterone concentrations. Earlier we have found that the drug at the dose of 500mg/kg increase corticosterone levels (Ara & Bano, 2009). Since depressed patients generally exhibit an elevated cortisol levels, the present data supports that part of the therapeutic properties of tianeptine, citalopram and SJW could be accounted for by the effects of these antidepressant to modulate the activity of the HPA axis and a possible decrease in circulating cortisol concentration in patients with depression would be an additional advantage of therapy with these antidepressants as this should limit excessive hepatic tryptophan degradation.

Plasma free tryptophan is an important peripheral parameter widely used by psycho pharmacologist to assess tryptophan entry into the brain for cerebral serotonin synthesis. We have found that all antidepressants increase brain tryptophan concentration by increasing free serum tryptophan, such an increase is independent of any effects of antidepressants on tryptophan pyrrolase activity. Tryptophan in plasma can be displaced from its binding site on albumin by non-esterified fatty acids (NEFA) and by certain drugs (Etienne *et al*., 1976). NEFA were not measured in present study. We have reported earlier that fluoxetine primary action in relieving depression is through elevation of serum tryptophan (free and bound) concentration by inhibiting tryptophan pyrrolase activity (Bano *et al*., 1999; Bano & Sherkheli, 2003; Bano *et al*., 2004). This improves tryptophan/LNAA ratio in favor of tryptophan resulting in enhance uptake by brain stimulating serotonin synthesis. In present study SJW significantly reduces the plasma total tryptophan levels, our findings are consistent with that reported by Yu (2000) but increases free tryptophan, the precursor of 5-HT. It is not clear whether increase in brain tryptophan with antidepressant is caused by tryptophan displacement, pyrrolase inhibition or both. Further work on tryptophan binding is therefore required.

A decrease in 5-HIAA after SSRIs and lack of increase in 5-HT is due to inhibition of 5-HT turnover almost certainly as a consequence of uptake inhibition. The increase in 5-HT and lack of increase in 5-HIAA after moclobemide is expected because of inhibition of MAO. Where as the lack of increase in 5-HT and 5-HIAA after tianeptine in the presence of increased brain tryptophan concentration, suggested other effects are caused by this drug such as regional increase in brain 5-HT such as brain stem, striatum and cortex as reported by Fattacini *et al.*, (1990) and Frankfurt *et al*., (1995). Other investigators have reported that tianeptine increases serotonin uptake in the brain (Wagstaff *et al*., 2001). A decrease in extracellular serotonin levels after tianeptine has been reported by Datla and Curzon (1993) in contrast in vivo microdialysis study have shown that single administration of tianeptine 10mg/kg did not change [5-HT (ext)] in the frontal cortex and raphe nuclei (Malagie *et al*., 2000) similarly we have also found no change in whole brain 5-HT and 5-HIAA levels. Present study also confirms that SJW increases 5-HT synthesis and turnover i.e. increased 5-HIAA levels and can act as an antidepressant. However present study supports the notion that it exhibits very

weak inhibitory activities towards MAO. An increase in 5-HT levels was also observed by Yu (2000) in hypothalamus and hippocampus. The increase of brain 5-HIAA was not further enhanced following chronic administration. It was suggested that the effects of SJW on brain 5-HIAA and 5-HT levels appears to be quite different from effects of classical 5-HT reuptake blockers. Calapai and coworkers (2001) reported that SJW at a dose 250-500 mg/kg with acute oral administration enhanced serotonin, norepinephrine and dopamine content in brain and reduced immobility time of rats in forced swim stress. Previously we (Bano & Dawood, 2008) have reported anxiolytic property of SJW on serotonergic modulations in swim stressed rats, in addition we have also found that SJW increases intra-neuronal 5HT metabolism but inhibits its release under adverse conditions proving its anxiolytic property (Ara and Bano, 2009).

Present work concludes that common mechanism of action of antidepressants of different classes and pharmacological profile is that of elevating brain tryptophan secondarily to increase in free tryptophan levels. These effects were independent of pyrrolase inhibition by antidepressants. It is also suggested that precursor availability plays key role for cerebral 5-HT synthesis, either for its increased release if required or metabolism.

ACKNOWLEDGEMENT

We thank British Commonwealth Commission for the visiting fellowship to S. Bano and Dean, Faculty of Science, University of Karachi for the Ph.D. research grant to Gitay M and Ara I.

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