INTRODUCTION

Many of the neurotransmitter substances are normal food constituents and directly modulate the brain biochemistry. Dietary components like glycomic and proteomic contents affecting the peripheral amino acid balance influence the availability of the amino acid L-Tryptophan (Trp) for the Central Nervous System (CNS).1,2 The synthesis of 5-hydroxytryptamine (5-HT; serotonin)2 occurs from its precursor L-Trp in neurons. The rate at which serotonergic neurons synthesize their 5-HT depends upon the availability of its precursor Trp. Trp is transported from the blood to the brain via an active uptake mechanism specific for Trp and other Large Neutral Amino Acids (LNAA) like valine (Val).2 Consequently, the ratio of Trp to the sum of LNAA (valine, leucine, isoleucine, tyrosine and phenylalanine) i.e. Trp:LNAA ratio reflects the useful concentration of Trp in the CNS.3,4 The administration of Trp or the consumption of glycomic rich diet/meal elevate brain Trp levels along with the levels of serotonin and its major metabolite 5-hydroxyindoleacetic acid (5-HIAA).2 Trp occurs in a low concentration (<1%) in most dietary proteomic sources. In order to get access to the brain, it must compete with other LNAA via a common cellular uptake mechanism. Low proteomic diets sway the ratio of Trp to LNAA in favour of Trp, so that more Trp is transported into the brain. Dietary supplementation of Trp can likewise increase the ratio of Trp to other LNAA, and afford Trp an advantage when vying for entry into the brain.3

A Branched-Chain Amino Acid (BCAA), L-Val competes with the Trp for transport into the brain and has previously been shown the decreased brain 5-HT synthesis.5 Conversely, it is reported that Trp load increases 5-HT synthesis in the brain and, therefore, may stimulate 5-HT release and functions.4,6 As a consequence of relations between plasma Trp/LNAA ratio, the ingestion of Val causes rapid elevation of their plasma concentrations, increases their uptake into the brain, and decreases the brain uptake and levels of the

ABSTRACT

Objective: To investigate the effects of orally supplemented amino acids L-Tryptophan (Trp) and L-Valine (Val) in rats repeatedly injected with haloperidol following one week of drug withdrawal, with particular reference to extrapyramidal symptoms (EPS) and serotonin (5-hydroxytryptamine; 5-HT) metabolism in medial prefrontal cortex (mPFC).

Study Design: Experimental study.

Place and Duration of Study: Department of Biochemistry, University of Karachi from December 2007 to February 2008.

Methodology: The study was conducted on thirty six locally bred male Albino Wistar rats. Freshly prepared amino acids (Val and Trp) were added in the drinking water of rats on alternate days and haloperidol at doses of 5.0 mg/kg or saline were injected twice daily for three weeks following one week of withdrawal. Locomotor/ exploratory activities were scored in activity boxes and open field apparatuses. Catalepsy was monitored on an inclined surface. The animals tested for locomotor activity and catalepsy for two weeks follow-up post-injections plus one week of drug withdrawal were decapitated to collect mPFC regions of rat brain for neurochemical analysis by high performance liquid chromatography with electrochemical detection (HPLC-EC).

Results: There was significant increase (p<0.01) in locomotor activity in rats orally supplemented with Val and Trp following one week of drug withdrawal from repeated administration. Marked reduction in cataleptogenic effects of the drug was also observed. Significant (p<0.01) increases in the brain Trp and mPFC 5-HT metabolism in Val and Trp supplemented animals were also noticed.

Conclusion: These findings help to demonstrate the effect of dietary amino acids, in particular, Trp to potentiate mPFC serotonergic modulation of neuroleptic activity.

Key words: Amino acids supplementations. Trp/LNAA ratio. Catalepsy. Haloperidol. mPFC-Serotonin. Schizophrenia.
The ability of antipsychotic drugs to modulate serotonergic as well as dopaminergic function has been suggested to be important for their efficacy and side-effect profile. Motor-related side-effects are commonly encountered in the treatment of schizophreniform psychoses with so-called “classical” antipsychotic drugs such as haloperidol that are known to block central dopamine (DA) receptors with their DA-D₂ antagonistic potential. However, there are interactions between dopaminergic and serotonergic neurons in the CNS, which may be of relevance to the catalepsy syndrome. There is also suggestive evidence for important DA/5-HT interaction in the mediation of EPS symptoms, as displayed in the catalepsy model in rats. It has been shown that stimulation of pre-synaptic 5-HT₁A, as well as postsynaptic 5-HT₂A/2C receptors may involve in the antagonism of catalepsy induced by dopamine receptor-blocking agents like haloperidol, suggesting a novel principle for attaining clinically effective antipsychotic blocking agents like haloperidol, suggesting a novel principle for attaining clinically effective antipsychotic agents with fewer or no EPS. It seemed pertinent, therefore, to investigate central 5-HT mechanisms in animals receiving chronic neuroleptic therapy in combination with oral Trp and Val supplementations. There is a possible importance of mPFC for cognitive, negative or positive symptoms of schizophrenia. Conversely, there has been relatively less study of the effects of antipsychotics with orally supplemented amino acid agents on the release of serotonin in the mPFC brain region. The mPFC is reported to have significant contributions of 5-HT₁A, 5-HT₁B, 5-HT₂A, 5-HT₃ and 5-HT₇ receptors. Therefore, the observed increases in extracellular 5-HT levels in the mPFC region can be expected to have significant effects on mesocortical 5-HT neurotransmission.

The present study was designed to test the effects of oral supplementations of amino acids (Val and Trp) on behavioural responses, plasma and brain mPFC Trp and mPFC 5-HT metabolism in rats treated with three weeks administration of haloperidol following one week of drug withdrawal. The results will possibly suggest the contribution of serotonin and its precursor amino acid Trp as adjuncts for the treatment of EPS symptoms of the neuroleptic agent haloperidol.

**METHODOLOGY**

Animals: Male Albino-Wistar rats with an average weight of 180±20 g on arrival, purchased from The Aga Khan University (AKU), were group-housed (two rats per cage) in an animal-keeping environmentally controlled room (ambient temperature 21±1°C and relative humidity 55±5%) on a 12:12-h light/dark cycle. A 5-day acclimatization period was allowed before animals were used in experiments. After this period, and 24 hours before the behavioural tests, the animals were individually housed in an environmentally controlled test room in transparent Perspex cages (dimensions 26 x 26 x 26 cm WxLxH). Standard rat diet and tap water were continuously available to animals during experiment. The rats used for the treatment were all experimentally naive animals. All experimental protocols were approved by and performed strictly in accordance with the international (US National Research Council, 1996) and the local ethical committee guidelines for animal research.

Drugs and Injections: Haloperidol (Serenaec, Seele, USA), purchased as injectable ampoules of 5 mg/ml, was injected in rats intraperitoneally (i.p.) at a dose of 5 mg/kg body weight twice daily between 9:00-10:00 a.m. and 3:00-4:00 p.m. hours for continuously 2 weeks. Amino acids Val and Trp, freshly prepared in tap water (200 ml measured volume) added in drinking water at a dose of 2 mg/ml (w/v) were administered orally for 3 weeks on alternate days. Control animals were injected with saline in volumes of 1 ml/kg body weight with oral administration of tap water at the same schedule.

Experimental Protocol: Animals of control (0.9% NaCl) and haloperidol (5 mg/kg) treated groups were equally supplemented with Val and Trp in their drinking water for continuously 3 weeks at a dose of 2 mg/ml freshly prepared in tap water. Washout period of the drug in the last week was taken as a measure to monitor withdrawal symptoms. Activities were monitored weekly in the familiar (home cage; in terms of number of cage crossings/ 10 minutes.) and novel (open field; in terms of latency to move in sec/ 5 minutes. and number of squares crossed/ 5 minutes) environments. Catalepsy, defined as the acceptance and retention of abnormal posture, was measured by means of a bar test. Bar test determinations were carried out by gently removing rats (n=12) from their home cages and placing their forepaws over a horizontal bar, fixed at a height of 10 cm with heads of animals upwards on an inclined surface at an angle of 60° with the hind limbs abducted. The length of time during which the animal retained this position was recorded by measuring the time from the placement of the rat until removal of one of its forepaws. Testing was performed 30 minutes post-injection of haloperidol after 2 weeks of treatment plus one week drug withdrawal, to monitor weekly changes on...
catalepsy and the time to withdrawal of legs by the rats was measured. A cut-off time of 180 seconds was employed to each rat in the treatment. Rats were removed from the bar if their latency on the bar test exceeded by 180 seconds. Effects on plasma and brain mPFC Trp, mPFC 5-HT and its metabolites were also determined in control and haloperidol treated rats with combined supplementation of Val and Trp for 3 weeks. Animals were decapitated after one week of withdrawal. mPFC brain regions were dissected out and immediately stored at −70°C for the determination of Trp and 5-HT metabolism by high performance liquid chromatography with electrochemical detection (HPLC-EC). Plasma samples were also stored for Trp determinations.15

Neurochemical Analysis: Dissection of Medial Prefrontal Cortex: Animals were decapitated and the brains were removed immediately from the cranial cavity as described by Batool and Haleem.4 The cerebellum was pinched out by forceps. The brain dipped in ice cold saline was placed with dorsal side up in the molded cavity of a brain slicer. A fine fishing line wire was inserted into the slots of the slicer to make 1 mm thick slices of brain. Desired brain regions were identified with the aid of a stereotaxic atlas. Olfactory nucleus material was discarded. Medial prefrontal cortices were dissected out with the help of sharp scalpel bilaterally and stored at −70°C in order to assay biogenic amines by HPLC-EC.

HPLC-EC determinations of plasma Trp, brain mPFC Trp and 5-HT metabolites: Brain samples were homogenized as described by Haleem,3 mPFC 5-HT and its metabolites were determined by HPLC-EC as described by Batool and colleagues.15 A 5 µ Shim-Pack ODS separation column of 4.0 mm internal diameter and 150 mm length was used. Separation was achieved by mobile phase containing methanol (14%), octyl sodium sulfate (0.023%) and EDTA (0.0035%) in 0.1 M phosphate buffer of pH 2.9 at an operating pressure 2000–3000 psi on Shimadzu HPLC pump. Electro-chemical detection was achieved on Shimadzu L-ECD-6A detector at an operating potential of 0.8 V (glassy carbon electrode vs. an Ag/AgCl reference electrode). Trp was determined in a separate run at an operating potential of 1.0V.

Statistical Analysis: Data on haloperidol-induced catalepsy, deficits of motor activity in novel and familiar environments were statistically analyzed by two-way ANOVA (repeated measure design) followed by Newman-Keuls test. The neurochemical and biochemical data on the effects of Val and Trp in repeated saline or repeated haloperidol injected animals were analyzed by two-way 2,3-ANOVA. Post-hoc comparisons were done with the Newman-Keuls test; p<0.05 were considered significant.

RESULTS

Figure 1 shows the effects of orally supplemented amino acids (Val and Trp) in rats treated with repeated administration (for 3 weeks) of haloperidol at a dose of 5 mg/kg and 2 weeks haloperidol plus one week of withdrawal on locomotor activity in a familiar environment (activity box; A) and (catalepsy; B).

There was non-significant V+T effects (F=1.4453; df=2,30; p>0.01), significant single drug effect (F=241.41; df=1,30; p<0.01) and insignificant interaction (F=2.395; df=1,30; p>0.01) between V+T and drug effects. Data obtained on locomotor activity for one week withdrawal showed significant V+T effect (F=28.011; df=2,30; p<0.01), non-significant single drug effect (F=2.9976; df=1,30; p>0.01) and significant interaction between V+T and drug effect (F=127.75; df=1,30; p<0.01).

Post-hoc analysis showed that repeated administration of haloperidol at a dose of 5 mg/kg significantly (p<0.01) decreased motor activity in rats treated with water, Val and Trp when compared with similarly treated saline injected rats. In contrast, following one week withdrawal effect of haloperidol, significant (p<0.01) increases were observed in both saline injected and haloperidol injected groups orally administered with Val and Trp from respective water treated rats. However, significant (p<0.01) decreases were observed in repeatedly injected haloperidol rats orally administered with water and Val from similarly treated saline injected rats.

Data obtained on catalepsy for repeated administration (for 3 weeks) of haloperidol at a dose of 5 mg/kg showed non-significant V+T effects (F=2.92; df=2,30; p>0.01), significant single drug effect (F=17120.1; df=1,30; p<0.01) and insignificant interaction (F=14.103; df=1,30; p>0.01) between V+T and drug effects. Data for one week haloperidol withdrawal showed significant V+T effect (F=6.5379; df=2,30; p<0.01), significant single drug effect (F=247.4; df=1,30; p<0.01) and significant interaction between V+T and drug effect (F=28.56; df=1,30; p<0.01).

Repeated administration of haloperidol at a dose of 5 mg/kg significantly (p<0.01) produced 100% catalepsy in rats orally supplemented with water, Val and Trp when compared with similarly treated saline injected rats. In contrast, following one week withdrawal effect of haloperidol, no 100% catalepsy was observed in haloperidol injected rats orally administered with water, Val and Trp when compared with similarly treated saline injected rats.

Figure 2 shows the effects following 6th day of withdrawal of haloperidol on open field exploratory activity (in terms of latency to move and number of squares crossed) in rats orally supplemented with water, Val and Trp. There was insignificant V+T effect
Farhat Batool, Shoaib Ahmed and Darakhshan Jabeen Haleem

(F=1.474; df=2,30; p>0.01), insignificant single drug effect (F=0.2687; df=1,30; p>0.01) and significant interaction between V+T and drug effect (F=76.55; df=1,30; p<0.01) and insignificant V+T effect (F=2.743; df=2,30; p>0.01), significant single drug effect (F=65.108; df=1,30; p<0.01) and significant interaction between V+T and drug effect (F=37.43; df=1,30; p<0.01) on 6th day of withdrawal from haloperidol respectively.

Open field exploratory activity showed significant (p<0.01) increases in exploratory activity following 6th day of withdrawal from haloperidol injections in rats orally supplemented with water, Val and Trp for 3 weeks when compared with respective water treated rats.

Figure 3 shows the effects of orally supplemented amino acids (Val and Trp) following one week of haloperidol withdrawal on plasma Trp, mPFC Trp and 5-HT metabolism in rats.

There was significant V+T effects (F=18.797; df=2,30; p<0.01), significant single drug effect (F=13.04; df=1,30; p<0.01) and significant interaction (F=7.68; df=1,30; p<0.01) between V+T and drug effects on plasma Trp. Significant V+T effects (F=14.651; df=2,30; p<0.01), non-significant single drug effect (F=0.7973; df=1,30; p>0.01) and significant interaction (F=25.162; df=1,30; p<0.01) were shown between V+T and drug effects on mPFC Trp. Post-hoc analysis on data of plasma Trp showed significant (p<0.01) increases in repeatedly saline injected rats orally supplemented with Val and Trp from respective water treated rats. However, significant (p<0.01) increases were also observed in repeatedly haloperidol injected group of rats orally supplemented with Val and Trp when compared with respective water treated rats. mPFC Trp showed insignificant (p>0.01) increases in repeatedly saline injected rats orally supplemented with Val and Trp from respective water treated rats. However, significant (p<0.01) increases of mPFC Trp were observed in repeatedly haloperidol injected group of rats orally supplemented with Val and Trp when compared with respective water treated rats.

There were significant V+T effects (F=466.68; df=2,30; p<0.01), significant single drug effect (F=31.04; df=1,30; p<0.01) and significant interaction (F=126.13; df=1,30; p<0.01) between V+T and drug effects on mPFC 5-HT. Data analyzed for mPFC 5-HIAA showed significant V+T effects (F=138.66; df=2,30; p<0.01), significant single drug effect (F=23.87; df=1,30; p<0.01) and significant interaction (F=26.23; df=1,30; p<0.01) between V+T and drug effects. Data analyzed for mPFC 5-HIAA/ 5-HT ratio showed significant V+T effects (F=26.91; df=2,30; p<0.01), significant single drug effect (F=23.387; df=1,30; p<0.01) and significant interaction (F=26.23; df=1,30; p<0.01) between V+T and drug effects.

Post-hoc analysis on data of brain mPFC 5-HT and its metabolites showed significant (p<0.01) increases in
Dietary amino acids effect on rat medial prefrontal cortex stimulation of somatodendritic receptors. The have reported that the anticataleptogenic effect was also amino acids with drug withdrawal effect. Other authors group of rats orally supplemented with Val and Trp when compared with respective water treated rats. However, significant (p<0.01) decreases were observed in repeatedly haloperidol injected group of rats orally supplemented with Val and Trp when compared with respective saline and water treated rats.

DISCUSSION
The typical effect of administering a dopamine receptor antagonist is a suppression of spontaneous exploratory locomotor behaviour and elicitation of a state known as catalepsy in animals. In the first part of the study, it was monitored that long-term treatment with postsynaptic dopamine DA-D2 receptor antagonist haloperidol at a dose of 5 mg/kg for 3 weeks produced maximal (100%) cataleptogenic effects in rats and the behaviour suppressing effects of haloperidol did not show tolerance with repeated dosing. However, oral supplementations of amino acids primarily Trp following one week of withdrawal from repeated administration of haloperidol exhibited significant increases in locomotor and exploratory activities and did not induce catalepsy. The effect of gradual daily withdrawal from long-term haloperidol on rat open field was also studied and it is recorded that haloperidol withdrawal induced a significant progressive increase in all parameters of activity (latency to move and exploratory activity) in rats. Results were considered to be a consequence of supersensitivity of central dopaminergic receptors. The effects of repeated administration of haloperidol are explainable in terms of either an increase in the responsiveness of postsynaptic 5-HT1A receptors or dopamine D2 receptors or both. It is suggested that augmented motor behaviours reflect an increase in the effectiveness of somatodendritic as well as postsynaptic 5-HT1A receptors following oral supplementation of amino acids with drug withdrawal effect. Other authors have reported that the ant cataleptogenic effect was also produced by the local application of 5-HT into the raphe region suggesting that the effects is produced by the stimulation of somatodendritic receptors. The results show that oral supplementations of amino acids and prior administration of haloperidol for 2 weeks plus one week withdrawal may be of help in the improvement of EPS induced by haloperidol. It is well-known that EPS of haloperidol can be counteracted by the administration of serotonergic agents. Furthermore, catalepsy or suppression in locomotor activity can be antagonized by Val and Trp supplementation as determined in the present study. The most important finding of present study is that we influenced the serotonergic system in the rat brain by means of Val and Trp supplementations. The results from the second part of the study showed that oral administration of Trp is followed by an increase in brain mPFC Trp concentration and consequently by an elevation in brain mPFC levels of 5-HT. These increases are more pronounced in groups of animals exposing to drug withdrawal episodes in the study. These findings concur with clinical reports. Thus, clinical improvement of chronic schizophrenic patients, in whom hyper functioning of central dopaminergic system has been hypothesized, was achieved by medication with precursor of 5-HT, L-Trp or 5-HTP. The levels of free Trp in plasma have been used as an index in the determination of brain Trp levels and oral supplementations of amino acids, primarily increased plasma and brain Trp augmented the release of 5-HT in the mPFC and an interaction between 5-HT and DA could partly account for this effect. The results indicate that the cataleptic effects of haloperidol depend on the balance between the dopaminergic and serotonergic systems, and that the serotonergic system exerts an inhibitory influence on the dopaminergic system.

These findings suggest a possible serotonergic involvement in neuroleptic induced short-term EPS and late appearing tardive dyskinesia and in amelioration of schizophrenia through Trp supplementations. Early studies have reported the use of a high dose (25 mg/kg) of haloperidol twice over a three-week interval in combination of a dietary Trp supplement. The combination of Trp plus haloperidol was found to cause a long lasting increase in spontaneous chewing movements. These observations are interpreted in the context of Trp supplementation to antipsychotic therapy. The results of the present study are in line with the view that repeated administration of haloperidol exerts a stronger effect upon the cortical serotonergic system and that this effect is mediated via 5-HT2 (possibly 5-HT2A) receptors. In this study, special attention was paid to the role of predominant 5-HT2 receptor blockade over D2 blockade. Whereas, D2 receptor blockade seems to be essential for the treatment of positive symptoms of schizophrenia, it also underlies in the induction of EPS. The present study shows time-dependent increases in 5-HT synthesis following the supplementation of amino acids with drug withdrawal effects in mPFC brain region. The increases were more marked in rats repeatedly injected with haloperidol plus oral Trp supplementations. An increase in the concentration of 5-hydroxyindoleacetic acid (5-HIAA), the primary metabolite of 5-HT was also observed in the mPFC of rats injected with haloperidol at a dose of 5 mg/kg in the present study. This interpretation can be further enlightened as Trp administration influences brain functions thought to be at least partly controlled by 5-HT neurons. Such effects can be blocked by co-administration of 5-HT antagonists and enhanced by co-injection of 5-HT reuptake blockers. These and other findings, thus strongly support the notion that Trp-induced increments in 5-HT.
synthesis enhance transmitter release and interaction with postsynaptic receptors. The effects of diet-mediated changes in brain Trp and 5-HT levels on these brain functions are interesting to elucidate. It is reported that oral administration of Trp-free amino acid mixture significantly decreased basal 5-HT and 5-HIAA levels 100 min. after ingestion (65 and 81% of basal value respectively) and remained at this level for another 140 minutes.9 These results thus show that removal of Trp from the balanced amino acid mixture decreased release of 5-HT in the rat brain. Other group of authors has reported that administration of Trp-free mixture of amino acids induced serotonin depletion in the rat frontal cortex.7 It is also reported that schizophrenia-like behaviour was exacerbated by the Trp-free mixture diet.7 The most interesting result of this study was that addition of Trp as a supplement to the chronic haloperidol treatment attenuates the catalepsy in rats with the concomitant rise in brain mPFC 5-HT synthesis. The important findings of the study support the idea that oral administration of amino acids in combination with haloperidol withdrawal from long-term administration augmented the uptake of Trp in the brain and consequently increased 5-HT synthesis in mPFC, which convincingly suggested that the rate of 5-HT formation varied directly with the availability of circulating precursor Trp levels, the source of which is dietary.

**CONCLUSION**

The present study showed that enhanced serotonergic neurotransmission in the brain mPFC plays an imperative role in improving EPS functions in rats. An increase in the effectiveness of somatodendritic as well as postsynaptic 5-HT1A receptors is one of the major contributing factors in the pathophysiology of schizophrenia and enhanced mPFC 5-HT metabolism in the present study elucidates the involvement of this region with intrinsic activity towards 5-HT2A receptors in schizophrenia and its etiology. The results further suggest that dietary supplementation of Trp as a nutraceutical adjunct to antipsychotic therapy or extracts of foods claimed to have a medicinal effect on mental health may be useful for the management of schizophrenia.

**Acknowledgement:** The authors would like to thank the Pakistan Science Foundation (PSF) and University of Karachi for a research grant.

**REFERENCES**


25. Liegeois JF, Ichikawa J, Meltzer HY. 5-HT_{2A} receptor antagonism potentiates haloperidol-induced dopamine release in rat medial prefrontal cortex and inhibits that in the nucleus accumbens in a dose-dependent manner. *Brain Res* 2002; 947:...