INHIBITION OF RESTRAINT-INDUCED ANOREXIA BY INJECTED TRYPTOPHAN

Darakhshan J. Haleem*, Bushra Jabeen and Tahira Parveen

Department of Biochemistry, Neurochemistry and Biochemical Neuropharmacology research unit, University of Karachi, Karachi 75270 Pakistan

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Abstract: Tryptophan injected at doses of 50mg/kg did not alter 24 h cumulative food intake and growth rate in rats. A single episode of 2 h restraint stress decreased food intake and growth rate of saline and tryptophan injected rats. The decreases of both food intake and growth rate were smaller in tryptophan injected (food intake 23.9% p<0.05; growth rate 2.9% p<0.05) than saline injected (food intake 78.5% p<0.01; growth rate 6.1% p<0.01) rats suggesting that tryptophan administration inhibits restraint-induced anorexia. Following an acute challenge with 2h restraint increases of 5-hydroxytryptamine (5-HT; serotonin) and 5-hydroxyindoleacetic acid (5-HIAA) but not tryptophan were greater in tryptophan injected than saline injected rats. The findings imply that tryptophan-induced increases of brain 5-HT and 5-HIAA have little effect on functional serotoninergic activity under basal conditions but a facilitatory effect on functional response occurs in conditions of increased serotoninergic neuronal activity such as during stress.

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Introduction

It is well known that the synthesis of 5-hydroxytryptamine (5-HT; serotonin) in the adult brain is dependent upon the availability of its precursor amino acid tryptophan to the serotoninergic neurons (1-3). This occurs because the rate limiting enzyme in the biosynthetic pathway of 5-HT, tryptophan hydroxylase, is unsaturated with its substrate under normal physiologic conditions (1). However, in view of lack of signs of behavioural activation normally associated with increased release of 5-HT in the brain, it is often questioned whether the increase in 5-HT turnover induced by the administration of tryptophan also leads to increased serotoninergic neuronal activity.

* To whom correspondence and reprint request may be addressed
A role of life event stresses in the precipitation of depression is known from many clinical surveys (4). Parallel studies on experimental animals show that an uncontrollable stress situation produces neurochemical changes and behavioural deficits, for example changes in ambulatory activity, food intake and growth rate, in experimental animals (5-8). Stress-induced behavioural deficits in experimental animals are widely used as models of depression (5-8). In a similar study we have previously reported that an episode of 2h restraint stress produced marked anorexia and decreased body weights in rats (9). Increases in whole brain and brain regional 5-HT metabolism and synthesis rate also occurred following an episode of 2-3 h restraint (7-10).

A role of 5-HT is described in both depression and anorexia. Classic hypothesis of 5-HT functions in depression describes 5-HT as an antidepressant compound (11) and a deficiency of 5-HT is described as the proximate cause of depression (12). Restraint-induced anorexia is an animal model of depression (6-9). Therefore, it appears that tryptophan-induced increases of 5-HT could attenuate restraint-induced anorexia. On the other hand, pharmacologic manipulations which tend to increase 5-HT functions in the brain are anorexiogenic and decrease food intake in experimental animals (13-15) suggesting that tryptophan-induced increases of 5-HT could potentiate restraint-induced anorexia.

The aim of the present study was to determine if tryptophan, the dietary amino acid precursor of 5-HT, could attenuate restraint-induced behavioural deficits in experimental animals. The effects of injected tryptophan are, therefore, monitored on restraint-induced anorexia in rats. In order to determine whether any effect of tryptophan if found on restraint-induced anorexia is due to brain 5-HT changes we have determined the effects of tryptophan on brain 5-HT metabolism in restrained and unrestrained rats. It was hoped that findings will provide an insight into the mechanism of antidepressant medication of tryptophan.

Materials and Methods

Animals and treatment:

Locally bred male albino Wistar rats weighing 200-220 g were housed individually under a 12 h light dark cycle (lights on at 6:00 h) in a quiet room with free access to standard rodent diet and water for at least 5 days before experimentation.

Experimental protocol:

Effects of tryptophan administration on daily cumulative food intakes:

Animals were randomly assigned to tryptophan injected and saline injected groups. Tryptophan was injected to the animals of respective group at doses of 50 mg/kg between 9:00 and 10:00 h daily for 4 days. Control animals were injected with saline at the same time. Food intakes were monitored daily between 8:00 and 9:00 h by weighing the food left in the hopper and calculated as g/100g body weight.

Effects of tryptophan administration on restraint-induced anorexia:

Animals were randomly assigned to 4 groups vis: saline injected unrestrained, saline injected restrained, tryptophan injected unrestrained and tryptophan injected restrained.
Tryptophan at doses of 50mg/kg and saline were injected between 9:00-9:30 h. Immediately after the injection animals assigned to restrained group were restrained on wire grids for 2h (8-9). Saline or tryptophan injected animals assigned to unrestrained groups were left unrestrained in their home cages during this period. Another injection of tryptophan or saline was made in the afternoon between 16:00-17:00 h. Food intakes and body weights were monitored next day between 9:00-9:30 h and calculated as g/100g body weight and percentage of initial day body weights (8-9).

Effects of tryptophan on brain 5-HT metabolism in restrained and unrestrained rats:

Animals assigned to saline injected unrestrained, saline injected restrained, tryptophan injected unrestrained and tryptophan injected restrained groups were injected with saline or tryptophan between 9:00-9:30 h. Immediately after injection the animals were restrained on wire grids (S-9) for 2h. Animals of the unrestrained groups were kept unrestrained in their home cages during this period. Immediately after the termination of restraint period animals were decapitated. Unrestrained animals were also decapitated at the same time. Brain removed within 30 sec were stored below -70°C for the estimation of tryptophan, 5-HT and 5-HIAA by HPLC-EC as described earlier (16). A 4um Novapak ODS 4.6 mm i.d. x 25 cm separation column was used. The solvent system was metanol(14%), octyl sodium sulphate (0.023%) and EDTA (0.0035%) in 0.1 M phsophate buffer. Electrochemical detection was performed at an operating potential of 0.8V (glassy carbon electrode vs Ag/AgCl reference electrode). Tryptophan was analyzed in a separate run using 25% methanol and an operating potential of 1.0 V.

Statistical analysis:

Data on the effects of 4 day administration of tryptophan on daily changes of food intakes were analysed by two way anova-repeated measure design. Effects of tryptophan on restraint-induced anorexia and body weight changes were analyzed by two way anova. Neurochemical data were also analyzed by two way anova. Post hoc comparisons were made by Newman-Keuls test. P values >0.05 were considered insignificant.

Results

Fig 1 shows daily changes of 24 h cumulative food intakes (calculated as g/100g body weight) in 4 day saline and 4 day tryptophan injected rats. Data analyzed by two way anova (repeated measure design) revealed insignificant treatment (F=0.49 df1,22) and day (F=1.18 df 4,88) effects. Interaction between two factors (F=0.66 df4,88) was also not significant.

Fig 2 shows the effects of 2 h restraint on 24 h cumulative food intakes (fig 2A) and growth rates (fig 2B) in saline and tryptophan injected rats. Two way anova (df1,20) revealed significant effects of stress on food intakes (F=19.5 p<0.01) and growth rates (F= 13.7 p<0.01). Effects of tryptophan administration on food intakes (F=4.2 p<0.01) and growth rates (F=7.1 p<0.01) were also significant. Interactions between stress and tryptophan were insignificant (food intakes F=2.18 p>0.05; growth rates F=1.9 p>0.05). Post hoc comparison showed that a single episode of 2 h restraint significantly decreased food intakes and growth rates in saline as well as in tryptophan injected rats. The decreases were smaller in tryptophan injected than saline injected...
rats. Therefore, mean values of food intakes and growth rates comparable in saline injected and tryptophan injected unrestrained animals were higher in tryptophan injected restrained than saline injected restrained rats.

Fig 1.
Effects of 4 day tryptophan administration at doses of 50mg/kg on daily changes of food intakes. Values are means ± SD (n=6). Differences by Two way anova repeated measure design were insignificant.

Fig 3 shows the effects of an episode of 2 h restraint on brain levels of tryptophan, 5-HT and 5-HIAA in saline and tryptophan injected rats. Two way anova (df 1,20) showed significant effects of stress on brain tryptophan (F=9.6 p<0.01; fig 3C), 5-HT (F=5.7 p<0.01; fig 3A) and 5-HIAA (F=42.8 p<0.01; fig 3B). Effects of tryptophan administration were also significant for tryptophan (F=110 p<0.01), 5-HT (F=26.3 p<0.01) and 5-HIAA (F=211 p<0.01) concentrations. Interactions between stress and tryptophan were insignificant for 5-HT (F=0.4 p>0.05) and 5-HIAA (F=0.3 p>0.05) but significant for tryptophan (F=4.9 p<0.05). Although 2 way ANOVA showed a significant effect of stress on brain 5-HT Individual comparison by Newman-Keuls test showed that restraint-induced increases of 5-HT in both saline and tryptophan injected rats were insignificant. Restraint-induced increases of tryptophan and 5-HIAA were significant in both saline and tryptophan injected rats. Tryptophan administration increased brain tryptophan, 5-HT and 5-HIAA concentrations in unrestrained rats. Values of 5-HT and 5-HIAA but not tryptophan were higher in tryptophan injected restrained than saline injected restrained rats.
Discussion

Administration of tryptophan increases brain 5-HT metabolism (1-3) because the rate limiting enzyme in the biosynthetic pathway of 5-HT, tryptophan hydroxylase, is unsaturated with its substrate (1). In the present study, similar to our previous study (3), 2 h after the administration of tryptophan at doses of 50 mg/kg, brain tryptophan 5-HT and 5-HIAA levels were significantly increased (fig 3). But tryptophan injected at these doses (50 mg/kg) daily for 4 days did not decrease 24 h cumulative food intakes (fig 1) in freely feeding rats. The decreases of food intakes were also not observed in rats injected with tryptophan at these doses two times a day and hence 24 h cumulative food intakes of saline injected unrestrained and tryptophan injected unrestrained rats were not significantly different (fig 2). The data suggest that increases of brain 5-HT metabolism that occur following the administration of tryptophan do not increase functional serotoninergic neuronal activity in normal freely feeding rats. These findings are consistent with studies which show that administration of tryptophan has little effect on extracellular serotonin release and hence serotoninergic activity under basal conditions (17-19).

Fig 2.
Effects of a single episode of 2 h restraint on A. 24 h cumulative food intakes (g/100g body weight) and B. growth rates (percent of initial day body weight) in saline and tryptophan (50mg/kg twice a day) injected rats. Values are means ± S. D. (n=6). Significant differences by Newman-Keuls test: *p<0.05, **p<0.01 from respective unrestrained rats, + p<0.01 from respective saline injected rats following two way anova.
The effects of 2h restraint stress on food intakes and growth rates of saline injected rats largely agreed with those of previous studies (7-9). Important finding of the present study is that tryptophan administration, although it did not alter food intake of freely feeding rats (fig 1), attenuated restraint-induced decreases of both food intakes and growth rates (fig 2). Behavioural deficits produced in experimental animals following an uncontrollable stressor are often described as model of depression (5-9) and in the present study these deficits were attenuated by the administration of tryptophan.

**Fig 3.**

Effects of a single episode of 2 h restraint on brain levels of A. 5-HT, B. 5-HIAA and C. tryptophan (50 mg/kg twice a day) in saline and tryptophan injected rats. Values are means ± S. D. (n=6). Significant differences by Newman-Keuls test: *p<0.05, **p<0.01 from respective unrestrained rats, +p<0.05, ++p<0.01 from respective saline injected rats following two way anova.

Restraint stress increases brain 5-HT turnover by increasing the availability of tryptophan to the brain (fig 3) has been also shown previously (7,10). In addition the present study shows that following 2h restraint brain levels of 5-HIAA and tryptophan are also increased in tryptophan injected rats while increases of 5-HT (fig 3A) and 5-HIAA (fig 3B) but not tryptophan (fig 3C) are greater in tryptophan injected than saline injected rats. A possible explanation for the lack of injected tryptophan’s effect in the enhancement of brain tryptophan concentration in restrained rats could be that in restrained rats a large amount of injected tryptophan is metabolized in the liver via kynurenine pathway (3). On the other hand, restraint-induced greater increases of brain 5-HT and 5-HIAA in tryptophan injected rats in the absence of significant increase in brain
tryptophan concentration may, in part, be a reflection of an increase in the activity of 5-HT synthesizing enzyme tryptophan hydroxylase during restraint (9).

Regardless of the mechanism by which restraint stress increases brain 5-HT and 5-HIAA concentrations more in tryptophan than saline injected rats, it is tempting to relate the attenuation of restraint-induced behavioural deficits (restraint-induced anorexia and decreases of growth rate) in tryptophan injected rats (fig 2) with the greater increases of brain 5-HT and 5-HIAA in tryptophan injected restrained than saline injected restrained rats (fig 3). Indeed, a deficiency of 5-HT is often described in human depression (11,12) and increasing 5-HT functions has antidepressant effect (20-22).

On the other hand, it is difficult to explain the attenuation of restraint-induced anorexia in tryptophan injected rats in terms of greater 5-HT increases because there is a great deal of pharmacologic evidence consistent with the view that serotonin contributes to the suppression of eating behaviour (13-15). Classic hypotheses describe 5-HT as an antidepressant as well as anorexiogenic compound. The present findings imply that tryptophan induced greater increases of 5-HT in restrained rats are adaptive and, therefore, attenuate restraint-induced anorexia.

In conclusion, the present study shows that tryptophan-induced increases of 5-HT do not increase functional serotoninergic activity under basal conditions possibly because the transmitter amine synthesized in extra amounts is not released to functional receptor sites (15). It would be interesting to monitor restraint-induced anorexia and levels of stress response proteins in rats given tryptophan rich diet. Regardless of the precise mechanism by which restraint-induced anorexia is inhibited by injected tryptophan, it is intriguing that tryptophan induced greater increases of brain 5-HT in restrained rats (fig 3) are adaptive and attenuate restraint-induced deficits (fig 2). The findings imply that this could be a mechanism by which tryptophan administration produces antidepressant effects. It is important to note that tryptophan’s availability can affect several neuronal processes in serotoninergic neurons. The lack of a functional response in any condition does not necessarily imply that serotonin is not involved in the mediation of response. It could be that in restrained rats tryptophan-induced serotonin release is more effective at sites involved in adaptation to stress than at sites involved in the suppression of appetite. The present findings are relevant that although increasing 5-HT functions is anorexiogenic. Depression and anorexia often coexist in humans (23-24). Food restriction decreases 5-HT content and synthesis rate in the hypothalamus of rat brain (16) but is suggested as the proximate cause of appetite suppression in anorexia nervosa (25).

References

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