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Research Article

Altered Brain Monoamines Metabolism in Thioacetamide Induced Hepatic Encephalopathy in Rats

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ABSTRACT

Altered monoamines metabolism has been reported in hepatic encephalopathy (HE). In order to evaluate this thioacetamide (TAA) was administered to induce hepatic encephalopathy due to fulminant hepatic failure in rats.

Methods: Female Albino Wistar rats (200-250 gm body wt) were selected and divided into two groups (n=6 in each group). Saline plus saline (controls) and TAA plus saline .TAA at a dose of 550 mg/kg was injected for two consecutive days in TAA plus saline treated rats. Brain concentrations of indoleamines, dopamine and their metabolites were measured by HPLC-EC. Brain concentrations of 5-hydroxy tryptamine (5-HT) were not significant. Whereas, its precursor Tryptophan (TRP) in the brain was significant ($p<0.05$), its metabolite 5-hydroxy indole acetic acid (5-HIAA) was significantly increased in TAA plus saline treated rats when compared to controls ($p<0.01$). Similarly concentrations of Dopamine (DA) were not increased in the brain. However, its metabolites dihydroxy phenyl acetic acid (DOPAC) and homo vanilinic Acid (HVA) was significantly increased in TAA plus saline ($p<0.01$). In plasma, TRP level was increased in TAA plus saline treated rats ($p<0.05$). Liver TRP levels were decreased in TAA plus saline treated rats when compared to controls but this decreased was insignificant. Liver total TRP and TRP pyrrolase apo enzyme activity were significantly decreased in TAA plus saline treated rats when compared to controls ($p<0.05$). Food intake was decreased on day 1 and on day 2 in TAA plus saline treated rats when compared to controls ($p<0.05$). Water intake was significantly decreased on day 1and on day 2 in when compared to controls ($p<0.01$). The results of this study show that an increased concentration of TRP, 5-HIAA and HVA, suggesting an increased turn over of 5-HT and DA in TAA plus saline treated rats.

Key words: thioacetamide, hepatic encephalopathy, tryptophan, tryptophan pyrrolase, serotonin, dopamine

INTRODUCTION

Hepatic encephalopathy (HE) is a complication of hepatic liver failure or a post systemic shunt surgery. Clinical features include confusion, which is often associated with a flapping tremor, drowsiness, stupor and coma. Repeated episodes of HE may lead to neuropsychiatry disturbances, dysarthria, ataxia and choreothetosis (1). An alteration in the metabolism of brain monoamines neurotransmitters have been proposed to be involved in the development of HE associated with experimental and human liver failure (2-4**)**. A role of indoleamine neurotransmitter serotonin (5-HT) is implicated in consciousness and sleep (5, 6). Increased levels of 5-HT, its precursor tryptophan (TRP) and levels of 5-hydroxy indole acetic acid (5-HIAA) in the brain have been reported in humans dying with HE and in animals dying with TAA induced Fulminant hepatic failure (FHF) (3,7,8).

The present study was designed to investigate the role of brain monoamines in the elicitation of Thioacetamide induced FHF in rats.

METHODS

The present study was conducted in the department of Biochemistry, University of Karachi, Pakistan. Prior approval was taken from the institutional ethical committee before the commencement of the experiment. Locally bred white female wistar rats weighing 200-250g, purchased from H.E.J institute of chemistry, University of Karachi were housed individually under a 12h light / 12h dark cycle (lights on at 06:00h) with free access to cubes of standard rodent diet and water for at least 3 days before experimentation. All experiments were conducted according to a protocol approved by Local Animal Care Committee.

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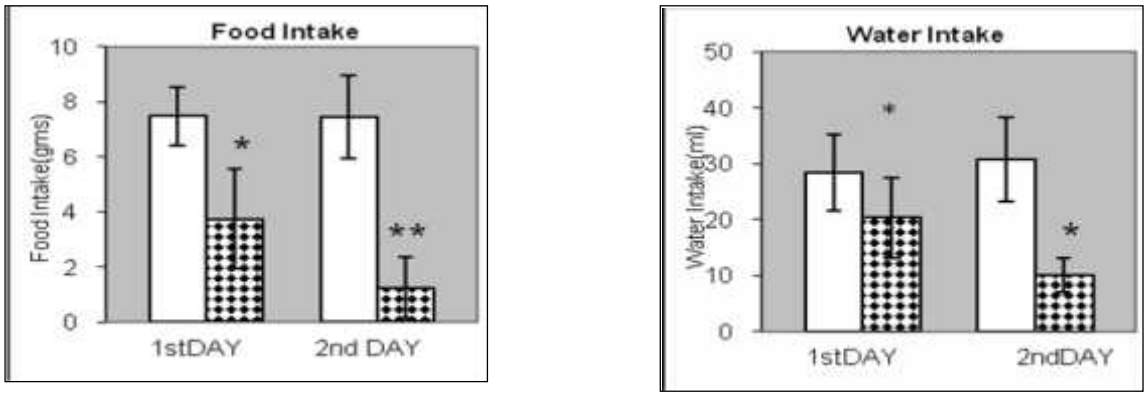

*Figure 1 Levels of food and water intake. Values are means ±S.D.(n=6).Significant differences by student's t-test.*p<0.05,**p<0.01 from respective controls .* control TAA

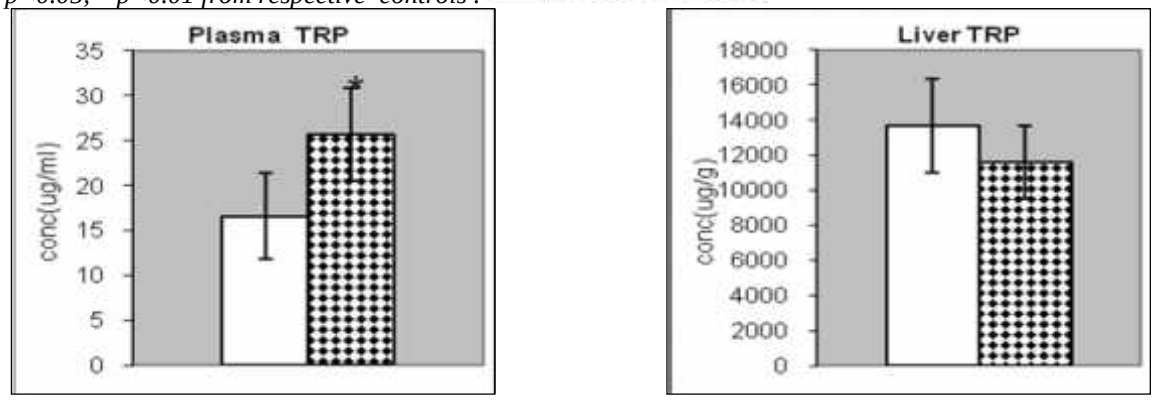

*Figure 2 Levels of plasma total and liver TRP. Values are means ±S.D.(n=6).Significant differences by student's t-test.*p<0.05 from respective controls .* control TAA

Drug administration: TAA (Sigma chemical Germany) at a dose of 550mg/kg was injected. The drug was dissolved in saline (0.9% NaCl) (10).

Experimental Protocol: Animals were randomly divided into two groups' i.e, Controls, and TAA plus saline treated groups. Each group had six rats. Weighed amount of food was placed in the hopper of cages of both groups of rats. Food intake and body weights were monitored daily. Fulminant hepatic failure (FHF) was induced in rats by intra peritoneal (i.p) injection of TAA (550 mg/5ml) saline/kg every 24 hours for 2 consecutive days (9). Control animals received an equal volume of saline (5 ml/kg). TAA plus saline treated rats received one injection of TAA (550 mg/5ml/kg) at 8:00 to 8:10 hours. Control animals received injection of saline (5ml/kg) at 8:10 to 8:20 hours and second injection of 1ml/kg of saline 30 minutes later. TAA plus saline also received injection of a solution containing 5 % dextrose in 0.45% NaCl, and 29 m eq/l of KCl 25 ml/kg, subcutaneous injection 12 hours after the first injection of TAA The behavioral manifestations of HE in the rat evolved through four stages: I, lethargy; II, mild ataxia; III, lack of spontaneous movement, loss of righting reflex, but positive response to tail pinch; and IV, coma, no response to tail pinch. Animals were decapitated after to the last injection by guillotine on the third day.

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 (2µm) hematin as described in detail by earlier researchers (10). The apo enzyme activity was obtained by difference (total enzyme activity– holo enzyme activity). Estimations of 5-HT, 5-HIAA and TRP and DA, DOPAC AND HVA concentrations in brain were determined by HPLC-EC. Liver and plasma TRP were also monitored by HPLC-EC. Brain samples were excised very quickly from the cranial cavity within 30 s of the decapitation. HPLC-EC determination was carried out according to standard procedure (11,12). A 5-II Shim-Pack ODS separation column of 4.0 mm internal diameter and150 mm length was used. Separation was achieved by a mobile phase containing methanol (14%), octyl sodium sulfate (0.023%) and EDTA (0.0035%) in 0.1 M phosphate buffer at pH 2.9 at an operating pressure of 2000–3000 psi on Schimadzu LEC 6A detector at an operating potential of 0.8 V for biogenic amines and1.0 V for TRP.

Behavioral tests

Food Intakes: Food intake was monitored by giving rats weighed amount of food and weighing the remaining food in the hopper of the cages.

STATISTICAL ANALYSIS

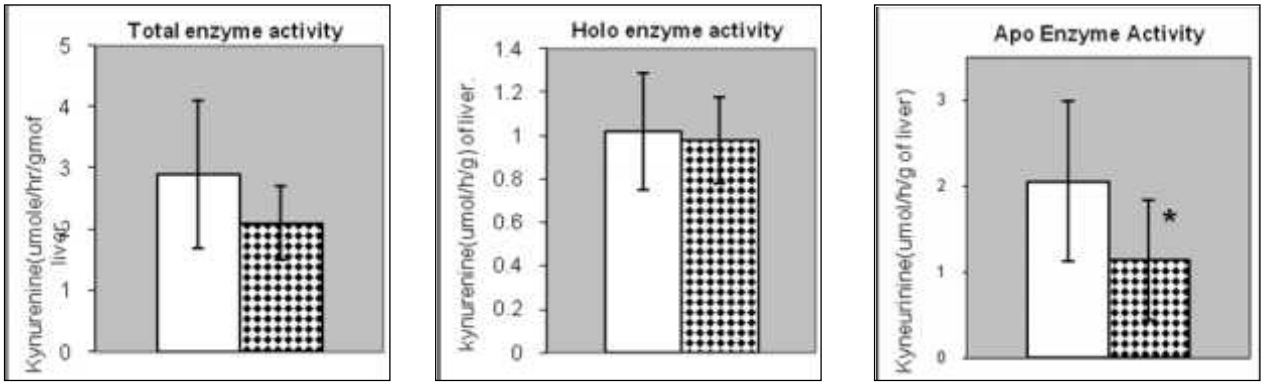

*Figure 3 Levels of total, holo and apo enzyme activity in liver. Values are means ±S.D.(n=6).Significant differences by by student's t-test.*p<0.05 from respective controls.* control TAA

The statistical significance of the results was analyzed by student's t-test. Differences between groups were taken significantly when p <0.05.

RESULTS

Effects of Taa Plus Saline on Food And Water Intake: Figure I shows the effects of TAA plus saline on 24 hours cumulative food (g) and water (ml) intake. Data analyzed by student's t-test showed a significant treatment effects on food intake on day 1and on day 2, ($p<0.01$). Effects on water intake were also significant ($p>0.05$) on day 1and on day 2.

Effects of taa plus saline on plama and liver trp concentration and hepatic try pyrrolase activity: Figures 2 and 3 show the effects of TAA plus saline on plasma and liver TRP concentration and on hepatic TRP pyrrolase activity. Data analyzed by student's t-test showed a significant increased ($p<0.05$) on plasma TRP .Liver TRP levels insignificantly decreased in TAA plus saline treated rats. Effects on holo enzyme activity were not significant. Whereas, total enzyme activity and apo enzyme activity were decreased significantly ($p<0.05$) in TAA plus saline treated rats.

Effects of taa plus saline on brain trp, 5-ht, 5-hiaa, da, dopac and hva concentrations: Figures 4 and 5 show the effects of TAA plus saline on brain TRP, 5-HT, 5-HIAA, DA, DOPAC and HVA concentrations. Data analyzed by student's t-test showed that TRP($p<0.05$) ,5-HlAA($p<0.01$), and DOPAC($p<0.01$) and HVA($p<0.01$), levels were significantly increased in TAA plus saline treated rats.

DISCUSSION

Altered monoamines neurotransmitter metabolism and functions are believed to underlie certain of the neuropsychiatry symptoms, e.g. depression, mania, and anxiety, encountered in clinical HE (13). In the present study TAA at a dose of (550mg/5m1 saline/kg) was administered for two consecutive days to induce HE in rats due to FHF (14). Onset of HE was monitored in TAA plus saline treated rats in four stages namely, Lethargy, mild ataxia, lack of spontaneous movement, and coma, (no response to tail pinch) (14).

The first significant decrease in behavioral manifestations in rats was observed on day 1 between 5 hrs and 6hrs in TAA plus saline treated rats. A significant decrease in food intake was observed on day 1and on day 2 in TAA plus saline treated rats. This finding is consistent with the previous findings which reported decreased ambulatory activity, food and drinking behavior in experimental models of hepatic encephalopathy (8, 15, 16). It has been reported earlier that rats with acute hepatic failure showed an increase in plasma TRP levels (8,14). In our previous study we have reported that patients with HE and cirrhosis exhibited an increase in the free fraction this amino acid rather than an increase in total tryoptophan (17, 18). However, in the present study an increase in plasma TRP occurred in TAA plus saline treated rats.

The availability of TRP in the circulation is dependent on the hepatic degradation of TRP (19). TRP pyrrolase activity was therefore determined in TAA plus saline treated rats. Almost 90% of blood TRP is catabolized in the liver. TRP pyrrolase (E.C1.13, 11.11) in the liver is the primary catabolizing enzyme of TRP (19, 20). Regulation of its action may influence plasma TRP levels (21). The activation of TRP pyrrolase in vitro consists of two steps. Inactive apo enzyme is first conjugated with heme to form oxidized holo enzyme. Oxidized holo enzyme is then reduced to the active form. Oxidation and reduction involves redox state of the heme (22). It has been shown earlier that activity of TRP pyrrolase is decreased in liver cirrhosis (23). The present findings show that activity of hepatic TRP apo enzyme is decreased significantly in TAA plus saline treated rats. Holo and total enzyme activity were not significantly decreased in TAA plus saline treated rats. The decreases of apo enzyme activity as observed in the present study are explainable in terms of decreased saturation of apo enzyme with its cofactor heme (24). In the present study liver TRP levels did not increase in TAA plus saline treated rats. More than 90% of the circulating TRP exists in protein bound form (25). The free fraction of plasma TRP is readily available to various tissues including liver and brain for utilization (26). Free fraction of TRP in the plasma was not monitored in the present study. However, an increase in plasma TRP concentration in TAA plus

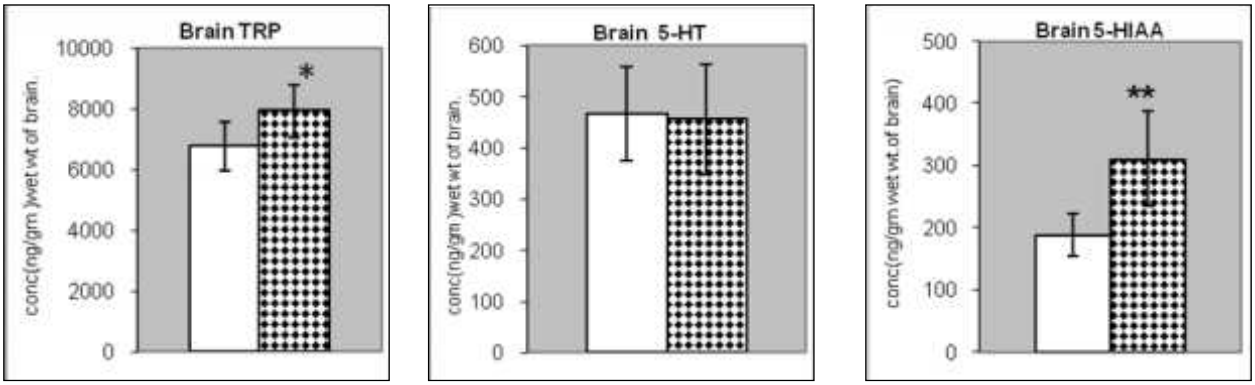

*Figure 4 Levels of TRP, 5-HT and 5-HIAA in the brain. Values are means + S.D (n=6). Significant differences by student's t-test.*p<0.05 ,**p<0.01 from respective controls.* control TAA

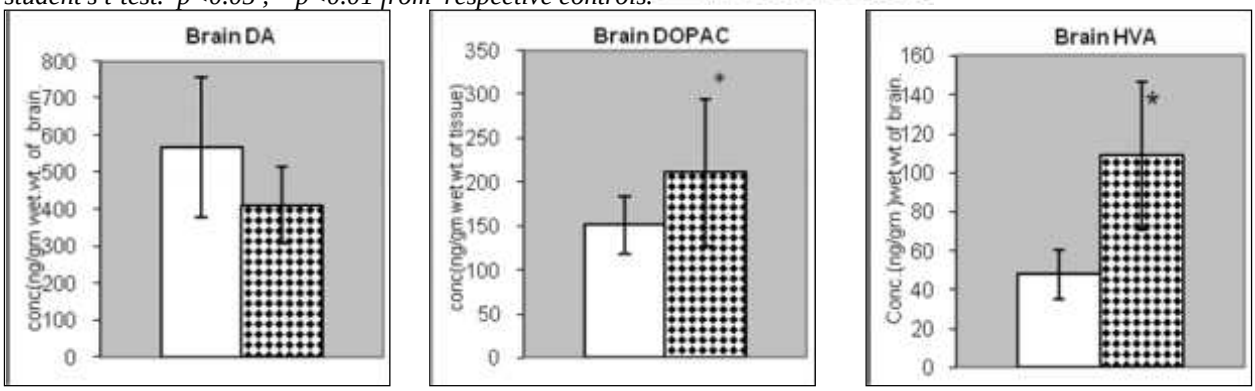

*Figure 5 Levels of DA, DOPAC and HVA in the brain. Values are means + S.D (n=6). Significant differences by student's t-test:*p<0.01 from respective controls.* control TAA

saline as observed in the present study could be occur because of a decrease in the activity of TRP pyrrolase.

Elevated levels of brain TRP have been reported in hepatic coma patients of acute (27) and CLD (28) in humans as well as in animals (8, 14).

Previous studies revealed that concentrations of AAAs (TRP, tyrosine, phenylalanine) are elevated in autopsied brain tissue of cirrhotic patients who died in hepatic coma (29) and in the brain (30) and CSF (31) of PCS rats. The present investigation shows an increase in brain TRP levels in TAA plus saline treated rats.

Increase in brain levels of 5-HT and 5-HIAA have been reported in the brain of PCS animals (8, 32) and in humans dying with HE. (3, 28). Previously it has been reported that 5-HT turn over instead of serotonin concentrations is increased in experimental liver diseases (18, 33). In the present study 5-HT levels did not increase in TA.A plus saline treated rats. Whereas, 5-HIAA levels were increased significantly in TAA plus saline treated rats. This increase in brain 5-HIAA in the absence of increased brain 5-HT levels in TAA plus saline treated rats suggests an increase in brain turn over of 5-HT.

It has been reported that hepatic encephalopathy in rats with D- galactose amine induced hepatic failure is associated with a decreased activity of the dopaminergic neurotransmitter system (34). It has been reported that DA content was unaffected however its metabolites were higher in the brain of an animal models of hepatic encephalopathy (4)

In the present study brain levels of DA did not increase in TAA plus saline induced HE in rats. Whereas, DOPAC and HVA levels significantly increased in TAA plus saline treated rats. Therefore, an increase in brain levels of HVA but not DA concentrations in the present study suggesting an increased turnover of DA in TAA plus saline treated rats.

CONCLUSION

To study the metabolism of TRP in fulminative drug induced encephalopathy in experimental animals the condition in rats was produced by the administration of TAA. An increase in total TRP concentration in plasma was expected to increase brain 5-HT. (35). However, we did not find an increased in brain 5-HT concentration.. An increase in concentration of brain TRP and 5- HIAA in the brain but not brain 5 HT suggesting an increased metabolism of 5-HT in TAA induced Fulminant Hepatic Failure (FHF) in rats.

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