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IS ANOREXIA IN THIOACETAMIDE-INDUCED CIRRHOSIS RELATED TO AN ALTERED BRAIN SEROTONIN CONCENTRATION?

Saida Haider[#], Sadia Saleem, Saima Shameem, Shahida P. Ahmed, Tahira Parveen, Darakhshan J. Haleem

Department of Biochemistry, Neurochemistry and Biochemical Neuropharmacology Research Unit, University of Karachi, Pakistan

Is anorexia in thioacetamide-induced cirrhosis related to an altered brain serotonin concentration? S. HAIDER, S. SALEEM, S. SHAMEEM, S.P. AHMED, T. PARVEEN, D.J. HALEEM. Pol. J. Pharmacol., 2004, 56, 73–78.

Anorexia or loss of appetite, one of the most typical symptoms observed in experimental and human cirrhosis, has been proposed to be associated with altered brain serotonin (5-HT) metabolism. In order to evaluate this hypothesis, brain 5-HT, its precursor tryptophan (TRP) and its metabolite 5-hydroxyindole-acetic acid (5-HIAA) were measured in brains of rats with thioacetamide (TAA)-induced liver cirrhosis. Thioacetamide at a dose of 500 mg/l in drinking water was administered for 6 weeks and during this period food intake was carefully measured in order to monitor the loss of appetite or decrease in food intake observed in cirrhosis. Concentrations of brain TRP, 5-HT and 5-HIAA were measured by HPLC with electrochemical detection. In TAA-treated rats, concentrations of 5-HT, TRP and 5-HIAA were increased in brain (44%, 33% and 36% of controls, $p < 0.01$). In plasma and liver of cirrhotic rats, TRP levels were increased (195% and 43%; $p < 0.01$). Plasma glucose and albumin levels were decreased (50%; $p < 0.01$ and 31%). Food intake, growth rate and locomotor activity of TAA-treated rats also decreased (73%, 22% and 73% of controls; $p < 0.01$).

The results of this study show that brain 5-HT concentration in rats is increased in TAA-treated rats and it may, therefore, play an important role in the pathogenesis of anorexia associated with TAA-induced cirrhosis.

Key words*: anorexia, brain serotonin, thioacetamide, cirrhosis*

[#] *correspondence;* e-mail: saidah13@yahoo.com

INTRODUCTION

Cirrhosis is a complex disease in which several biological and biochemical alterations are combined. It is characterized anatomically by widespread nodules in liver combined with fibrosis. The fibrosis and nodule formation causes distortion of the normal liver architecture which interferes with blood flow through liver. Cirrhosis can lead to an inability of liver to perform its normal functions. As disease progresses the symptoms become serious. The most common complication of the disease is hepatic encephalopathy. Two of the early common symptoms of liver cirrhosis are weight loss and loss of appetite [3, 20, 25, 26].

A role of a neurotransmitter serotonin (5-HT) in the regulation of food intake is well established [8, 12]. It has been suggested that changes in brain tryptophan (TRP) and, hence, brain 5-HT may convey information to the brain about nutritional state of the organism [7, 8]. A number of studies have shown that increasing 5-HT functions in the brain decreases food intake in experimental animals [7, 28]. Increased availability of TRP to the brain has been previously associated with anorexia in cirrhosis [10, 20].

The present study was designed to investigate the possible relationship between brain 5-HT concentration and decrease in food intake (anorexia) in thioacetamide (TAA)-induced cirrhosis in rats.

MATERIALS and METHODS

Animals

Locally bred white female Wistar rats weighing 200–250 g, purchased from H.E.J Institute of Chemistry were housed individually under a 12 h light/12 h dark cycle (lights on at 06:00 h) and controlled room temperature (22 ± 2°C) 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.

Drug administration

TAA (Sigma Chemicals, Germany), was given orally in drinking water at a dose of 500 mg/l daily for 6 weeks.

Experimental protocol

Animals were randomly divided into control and test groups. Weighed amount of food was placed in the hopper of cages of both groups of rats. Food intakes and body weights of both groups of rats were monitored weekly. Control rats were given tap water while test rats were given TAA (500 mg/l) in drinking tap water. Growth rates were calculated as percentage changes in body weights, [(body weight/starting week body weight) × 100] as described previously [13]. Ambulatory activities of both control and TAA-treated rats were monitored weekly. After 6 weeks of treatment the rats were decapitated between 10:00 and 11:00 h to collect plasma and brain samples. Whole brains were stored at –70°C until analysis of indoleamines by HPLC-EC as described before [16]. Liver, plasma and brain TRP levels were also determined by HPLC-EC. Plasma glucose and albumin levels were determined by standard laboratory procedures.

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.

Growth rate

Body weights of rats were monitored weekly during the 6 weeks of treatment. Growth rate was calculated in terms of percentage of initial body weight.

Locomotor and exploratory activity

The activity of control and TAA-treated rats were monitored weekly in an open field apparatus which consisted of a square area measuring 76 × 76 cm with walls 42 cm high. The floor was divided by lines into 25 equal squares. To determine activity, a rat was placed in the central square of the open field and the number of squares crossed with all four paws was scored for 5 min as described earlier [15]. Activities of control and TAA-treated animals were monitored in a balanced design to avoid order effect.

Statistical analysis

The statistical significance of the results was computed by Student's *t*-test (unpaired two tailed); p values < 0.05 were taken as significant.

RESULTS

Induction of liver cirrhosis

TAA which is an adequate animal model of cirrhosis was used to induce cirrhosis in rats. Induction of cirrhosis was confirmed by anatomical and histological studies.

The effects of 6 weeks of TAA administration on livers of control and TAA-treated rats

Figure 1 shows the effects of 6 weeks of oral administration of TAA (500 mg/l) in drinking water on rat liver. Cirrhotic rat liver (A) shows the development of reddish black granulation on the surface and uneven boundary. Normal liver is shown in Figure 1B. The histopathological evaluation of liver sections from TAA-treated rats showed necrosis, destruction of the lobule architecture and proliferative changes characteristic of cirrhosis.

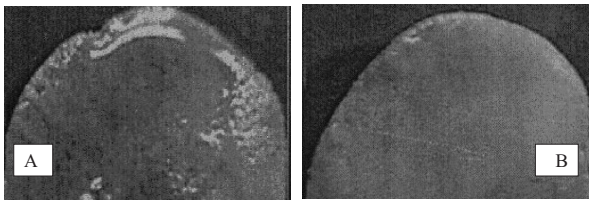

Fig. 1. Photographs of whole livers from TAA-treated (A) and control (B) rats showing the presence of uneven boundary and reddish black nodules on the surface of the liver of TAA-treated rats. Photograph taken 6 weeks after treatment with TAA

The effects of 6 weeks of TAA administration on weekly food intake, body weight and open field activity in control and TAA-treated rats

Data analyzed by Student's *t*-test revealed a significant decrease in food intake (Fig. 2A) and growth rate (Fig. 2B) starting from the second week onwards. Weekly changes of food intake and growth rate were not different in control rats, however, in TAA-treated rats values which were not different after first week, decreased significantly after second week. At the end of 6th week, there was almost a 73% decrease in food intake and 22% reduction in growth rate of TAA-treated rats. A significant decrease ($p < 0.01$; 73%) in locomotor activity (Fig. 2C) of TAA-treated rats was also observed.

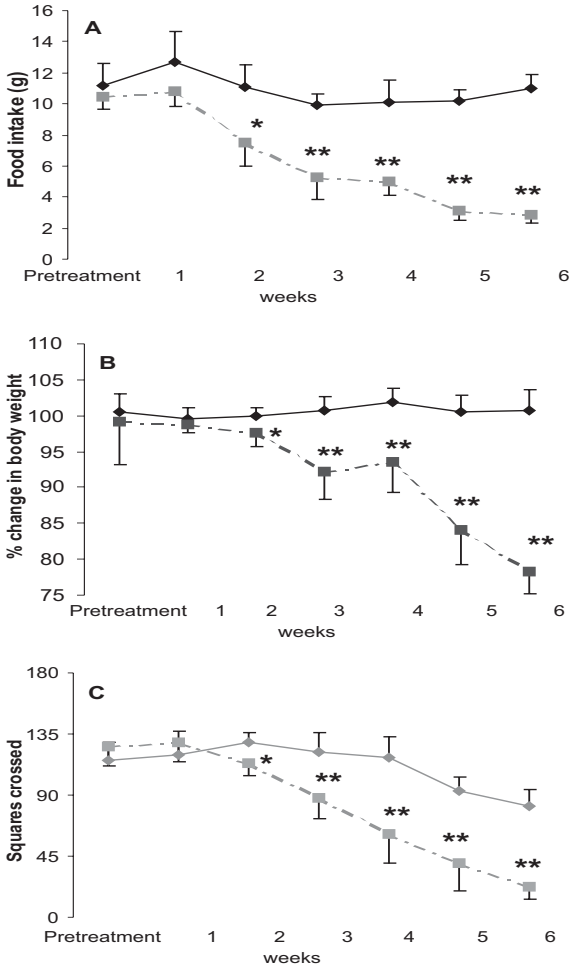

Fig. 2. Weekly food intakes (g) (A), % change in body weight (B), and open field activity (C) of controls (smooth line) and TAA-treated (dashed line) rats. Values are means ± SD (n = 6). Significant differences by Student's *t*-test; *$p < 0.05$, **$p < 0.01$ *vs*. respective week-water treated rats

The effects of TAA-induced cirrhosis on plasma glucose (A) and plasma albumin (B) levels in control and TAA-treated rats

Data analyzed by Student's *t*-test showed a significant decrease by almost 50% in glucose levels in TAA-treated rats. Plasma albumin levels exhibited a 31% decrease in TAA-treated rats (Fig. 3).

The effects of TAA-induced cirrhosis on liver (A) and plasma (B) TRP levels in control and TAA-treated rats

Data analyzed by Student's *t*-test revealed a significant ($p < 0.01$) increase in both liver (43%) and plasma (195%) TRP concentrations (Fig. 4).

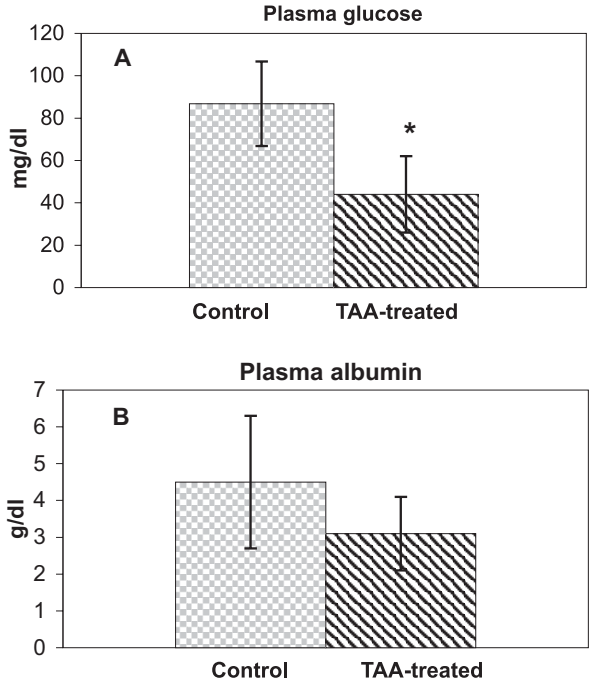

Fig. 3. Plasma glucose (mg%) (A) and plasma albumin (g%) (B) in controls and TAA-treated rats. Values are means ± SD (n = 6). Significant differences by Student's *t*-test; *p < 0.01 *vs*. respective water-treated rats

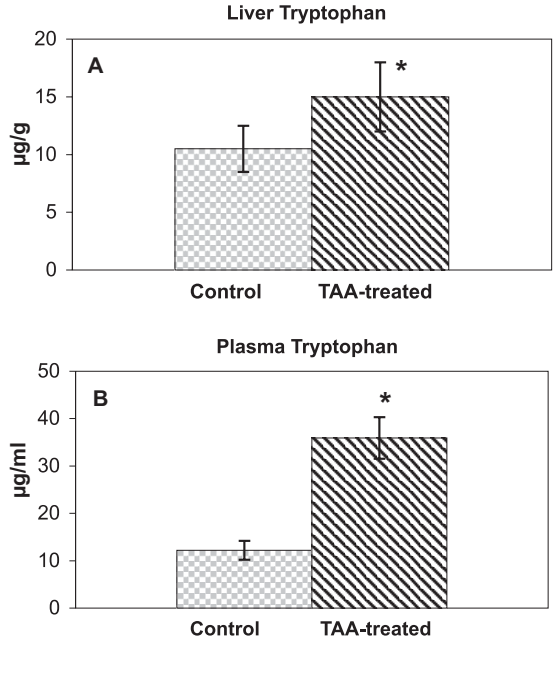

Fig. 4. Liver tryptophan (µg/g) (A) and plasma total TRP (µg/ml) (B) in controls and TAA-treated rats. Values are means ± SD (n = 6). Significant differences by Student's *t*-test; *p < 0.01 *vs*. respective water-treated rats

The effects of TAA-induced cirrhosis on brain tryptophan, 5-HT and 5-HIAA in control and TAA-treated rats

Data analyzed by Student's *t*-test showed a significant increase in brain TRP (33%, p < 0.01), 5-HT (44%, p < 0.01) and 5-HIAA (36%, p < 0.01) levels (Fig. 5).

DISCUSSION

Anorexia and weight loss are two important symptoms observed in cirrhosis [3, 20, 22]. In the present study, TAA was used to induce cirrhosis in rats. Oral administration of TAA at a dose of 500 mg/l in drinking water for 6 weeks induced cirrhosis in rats. Induction of cirrhosis was confirmed by anatomical and histological study of TAA-treated livers. A gradual decrease in food intake and growth rate was observed in TAA-treated rats. The decreases were significant from the second week continuing onwards till the end of the experiment. At the end of the experimental period, the decrease in food intake and growth rate was 73% and 22%, respectively, compared to water-treated controls. This finding is consistent with the previous findings which reported decreased food intake or anorexia in cirrhosis [3, 20, 25].

Increased transport of TRP into the brain has been reported in various liver diseases [10, 20, 21]. This increased brain TRP availability has been associated with anorexia in liver cirrhosis. Patients with hepatic encephalopathy and cirrhosis are reported to exhibit an increase in the free fraction of the amino acid rather than an increase in total TRP [29]. However, after hepatectomy [11] as well as after 2,4 dimethylnitrosamine poisoning [3] abnormally high concentration of plasma total TRP were also measured. Therefore, the elevated levels of plasma total TRP in the present study is an acceptable finding.

The availability of TRP in the circulation is dependent on the hepatic degradation of TRP. Almost 90% of blood TRP is catabolized in the liver by enzyme TRP pyrrolase [4]. It has been shown earlier that activity of TRP pyrrolase is decreased in liver cirrhosis [26]. Increases in plasma and liver TRP in cirrhotic rats is, therefore, explainable in terms of decreased activity of TRP pyrrolase in the liver. Elevated levels of brain TRP have been reported in

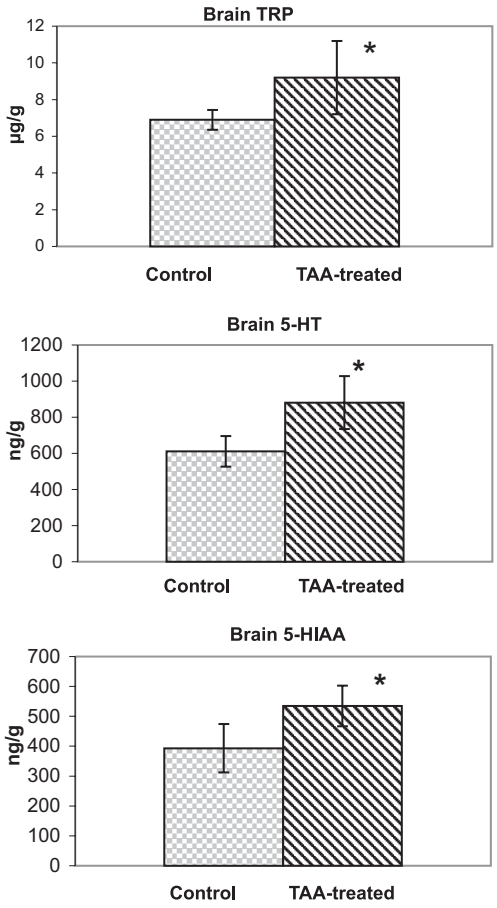

Fig. 5. Brain TRP (µg/g) (A), 5-HT (ng/g) (B) and 5-HIAA (ng/g) (C) in controls and TAA-treated rats. Values are means ± SD (n = 6). Significant differences by Student's *t*-test; *p < 0.01 *vs*. respective water-treated controls

hepatic coma patients with acute and chronic liver diseases in humans [18] as well as in animals [5]. The present finding also shows an increase in brain TRP levels.

Role of 5-HT and TRP in the regulation of food intake is well established [7–9]. Pharmacological research has suggested that anorexia observed in various liver diseases may be due to an increase in the availability of TRP to the brain which leads to a rise in brain 5-HT synthesis which in turn sends a neurochemical signal for the termination of meal [27, 28]. In this regard, the interesting finding of the present study is the significant increase in brain 5-HT concentration of TAA-treated rats. In various other animal models that is after hepatectomy [5, 29], liver devascularization [10] or carbon tetrachloride treatment [19], either normal or near normal concentrations of brain 5-HT are reported. In view of increased levels of 5-HIAA [17, 18], it has generally been assumed that only 5-HT turnover instead of serotonin concentration is increased in experimental liver diseases [2, 29]. However, in the present study, both 5-HT and 5-HIAA concentrations were increased in the brains of TAA-treated rats. The decrease in food intake observed in the present study may be attributed to the increase in brain 5-HT concentration. A number of studies have shown that manipulations which tend to increase 5-HT functions in the synaptic cleft decrease food intake of experimental animals [7, 14]. Clinical reports suggest that this decrease is due to the suppression of appetite. The results of the present study, therefore, indicate a relationship between increased brain 5-HT concentration and loss of appetite in TAA-induced cirrhosis in rats. Anorexia is said to occur in metabolically severe cirrhosis. In the present study, low plasma glucose (50%) and albumin (31%) concentrations point to a marked impairment of liver function in TAA-treated rats.

The neurotransmitter 5-HT has a profound effect on the control of sleep [24]. Administration of 5-HT leads to behavioral and electrophagic evidence of sleep while inhibition of 5-HT synthesis leads to a reduction in sleep and brain 5-HT concentration [1, 30]. Alteration in the metabolism of monoamines have also been observed in both humans and animal models of hepatic encephalopathy [23, 31]. A reduction in spontaneous locomotor activity was observed in operated, sham operated and non operated rats with portacaval shunt [6]. In the present study, the locomotor activity of cirrhotic rats decreased significantly. This decrease in activity of TAA-treated rats may be attributed to the increased serotonin activity in brain of these rats.

In conclusion, the present findings show that the metabolism of brain 5-HT is increased in TAA-induced cirrhosis. An increase in plasma tryptophan concentration in patients with various liver disorders has been shown in clinical studies [10, 20, 25]. The present study shows that plasma tryptophan levels also increase in experimental TAA-induced cirrhosis in rats. It shows that the increases of plasma TRP in cirrhosis and possibly also in other liver disorders could increase 5-HT synthesis in the brain. The resultant increase in 5-HT functions may lead to a decrease in food intake as observed in the present study in rats and in other clinical studies on liver cirrhosis. Previously in cirrhosis and other liver disorders, enhanced

availability of 5-HT precursor TRP to the brain was associated to the anorexia observed in these diseases. In the present study, increased brain 5-HT concentration and the simultaneous decrease in food intake in TAA-treated rats confirm the involvement of brain 5-HT in the pathogenesis of anorexia observed in the TAA-induced liver cirrhosis.

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