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Hoe 140 abolishes the blood pressure lowering effect of taurine in high fructose-fed rats

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Summary. High fructose feeding induces moderate increases in blood pressure of normal rats, associated with hyperinsulinemia, insulin resistance and impaired glucose tolerance. Increased vascular resistance, and sodium retention have been proposed to contribute to the blood pressure elevation in this model. Taurine, a sulphur-containing amino acid has been reported to have antihypertensive and antinatriuretic actions. In addition, taurine is shown to increase the excretion of nitrite and kinin availability and hence would be expected to improve the vascular tone. In the present study, the involvement of kinins in the blood pressure lowering effect of taurine was investigated by coadministration of Hoe 140, a kinin B_2 receptor antagonist along with taurine. The effects of taurine on plasma and urinary concentrations of sodium and tissue kallikrein activity were studied in high fructose-fed rats. Fructose-fed rats had elevated blood pressure and decreased levels of sodium in urine. Treatment with 2% taurine in drinking water prevented the blood pressure elevation and coadministration of Hoe 140 abolished this effect of taurine in high fructose-fed rats. The findings confirm the antinatriuretic action of taurine and also suggest a role for the kinins in the mechanism of taurine action in diet-induced hypertension.

Keywords: Fructose feeding – Hypertension – Kallikrein – Sodium – Taurine

Introduction

High dosage of fructose exerts a number of adverse metabolic alterations in experimental animals including hypertriglyceridemia, hyperinsulinemia and glucose intolerance (Thornburn et al., 1989; Buchanan et al., 1992). Fructose feeding also induces a moderate increase in blood pressure which is associated with a series of changes such as volume overload, sodium retention by kidneys and increased sympathetic activity among others (Hwang et al., 1989).

Kinins are potent vasodilatory and blood pressure lowering peptides, with additional effects on renal handling of electrolytes and water (Katori and Majima, 1999). Kinins are formed in tissues by the enzymatic action of kallikreins on kininogens. Kinins are reported to affect water and electrolyte homeostasis by acting on the vascular, interstitial and tubular compartments of the kidney (Carretero et al., 1991).

Taurine (2 amino ethane sulfonic acid) is a major free amino acid in the plasma and is widely distributed in animal tissues, including nervous tissue, heart, liver, kidney and retina (Yokogoshi et al., 1999). Apart from its role in bile acid conjugation in liver, taurine functions as a growth factor, cytoprotective agent, inhibitory neuromodulator and regulator of intracellular calcium concentrations (Huxtable, 1992; Timbrell et al., 1995). Taurine comprises over 50% of the total free amino acid pool of the heart and has a positive inotropic action on cardiac tissue. Taurine's actions have been postulated to stabilize and conserve function in an unstable physiological system, a property termed enantiostasis (Huxtable, 1992).

In a previous study we reported that taurine attenuates the rise in blood pressure in high fructose-fed rats (Anuradha and Balakrishnan, 1999). We now report the possible participation of the kallikrein-kinin system in the regulation of blood pressure and electrolyte balance by taurine in fructose-fed rats. The influence of co-treatment of the specific bradykinin B2 receptor antagonist HOE 140 (D-Arg (Hyp^3, Thi^5, D-Tic^7, Oic^8) – bradykinin: Thi, thienyl-alanine; Tic, 1,2,3,4-tetrahydroisoquinoline-3- carboxylic acid; Oic, [3as, 7as]- octahydroindol-2 carboxylic acid) with taurine on blood pressure was examined.

Materials and methods

Animals

Male adult Wistar rats of body weight ranging from (170–190 g) were obtained from the Central Animal House, Rajah Muthiah Medical College,

Annamalai University. They were housed two per cage under controlled conditions on a 12 h light/12 h dark cycle. They all received a standard pellet diet (Karnataka State Agro Corporation Ltd, Agro feeds division, Bangalore, India) and water *ad libitum*.

After acclimatisation the animals were divided into the following groups consisting of 12 rats each.

Experimental groups

Group 1 – Control animals (CON) received the commercial diet containing vegetable starch (61%) as the sole source of carbohydrate and tap water *ad libitum*.

Group 2 – Fructose-fed animals (FRU) received a fructose-enriched diet containing 60% fructose, 20% casein, 0.7% methionine, 5% groundnut oil, 10.7% wheat bran and 3.5% salt mixture and water *ad libitum*. 0.2 ml of vitamin mixture [Vitamin A concentrate I.P., 2500 I.U; Vitamin D_3 Cholecalciferol, 200 I.U; Thiamine hydrochloride, 0.5 mg; Riboflavin, 0.5 mg; Pyridoxin, 0.5 mg; Sodium pantothenate, 1.5 mg; Nicotinamide, 5 mg; Ascorbic acid, 25 mg. (ABDEC multi vitamin drops, Pharmpak pvt. Ltd., Mumbai)] was added per kg feed. The diet was prepared freshly every day.

Group 3 – Fructose-fed animals (FRU-TAU) received the fructose diet and were allowed to drink 2% taurine solution *ad libitum*.

Group 4 – Control animals (CON-TAU) received the commercial diet and were given 2% taurine solution *ad libitum*.

The animals were maintained in their respective groups for 30 days. Food intake, fluid intake and body weight changes were measured regularly. 24 h urine samples were collected during the end of the experimental period.

Blood pressure measurement

A set of six animals from each group were used for the measurement of systolic pressure at the end of the 2nd week and 4th week using the direct catheterization method as described earlier (Balakrishnan and Anuradha, 1997).

In order to test the involvement of kinins in the blood pressure lowering effect of taurine a kinin B_2 receptor blocker HOE 140 was co-administered along with taurine at a dose of 100 μg/day/kg s.c. for 7 days and blood pressure was measured in these rats.

Biochemical analysis

At the end of 30 days the animals were anesthetized using light ether and sacrificed by cervical decapitation. Blood was collected and plasma was separated by centrifugation and processed for determination of sodium and kallikrein. Heart and kidney dissected out and washed in ice-cold saline and weighed.

Kallikrein activity in plasma, urine, kidney and heart was assayed using a synthetic substrate benzoyl L-arginine ethyl ester at a final concentration of 1 mM in pyrophosphate semicarbazide glycine buffer (pH 8.7) containing soybean trypsin inhibitor (Trautschold et al., 1974). Plasma and urinary sodium were also measured (Natelson, 1957).

Statistical analysis

Values are given as means ± SD. The differences between groups were analysed using ANOVA followed by Duncan's test. The level of statistical significance was set at $p < 0.05$.

Results

The initial and final body weights, fluid and food intake of the animals and the urinary volume during the experimental period are given in Table 1. Body weight gain was similar for all groups and the final body weight were not significantly different from each other. Food intake during the experimental period did not vary among the various groups. Fluid intake by the animals in FRU-TAU and CON-TAU groups was higher than that of the CON animals. The urine volume was higher in FRU-TAU and CON-TAU groups than that of the controls.

The blood pressure changes are presented in Table 2. Systolic blood pressure of fructose-fed rats was significantly higher than that of the controls. Blood pressure values were significantly lower in fructose-fed animals treated with taurine as compared to fructose-fed rats. There was no significant alteration in blood pressure values

Table 1. Body weight, food intake, fluid intake and urine volume of control and experimental animals

	CON	FRU	FRU-TAU	CON-TAU
Body weight (gms)				
Initial	174.83 ± 1.72	177.33 ± 2.41	176.50 ± 1.98	174.67 ± 2.92
Final	178.33 ± 1.97	182.83 ± 4.81	181.33 ± 3.24	178.50 ± 2.63
Food intake (g/kg b.w/day)				
2nd week	91.1 ± 9.2	93.2 ± 6.8	89.4 ± 7.2	86.4 ± 5.6
4th week	83.8 ± 11.2	84.9 ± 10.1	85.2 ± 9.2	86.4 ± 5.6
Fluid intake (ml/kg b.w/day)				
2nd week	135.8 ± 10.2	133.5 ± 12.2	138.5 ± 15.2	136.5 ± 18.2
4th week	183.3 ± 13.6 [a]	188.8 ± 15.6 [a]	197.5 ± 15.2 [b]	200.7 ± 15.1 [b]
Urine volume (ml/day/100 g b.w)				
2nd week	7.17 ± 0.70 [a]	8.21 ± 0.54 [b]	9.15 ± 0.22 [c]	9.51 ± 0.33 [c]
4th week	7.36 ± 0.37 [a]	8.39 ± 0.54 [b]	9.17 ± 0.64 [c]	9.54 ± 0.23 [c]

Values are mean ± SD of 6 rats from each group
Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT)

Table 2. Blood pressure and heart weight/body weight ratio of control and experimental animals

	CON	FRU	FRU-TAU	CON-TAU
Blood pressure (mmHg)				
2nd week	95.67 ± 1.37 [a]	137.33 ± 4.80 [b]	98.5 ± 1.87 [a]	98.78 ± 1.72 [a]
4th week	113.00 ± 2.37 [a]	142.00 ± 2.61 [b]	111.0 ± 2.0 [a]	110.33 ± 1.63 [a]
Heart weight/body weight ratio (mg/100 g)	367.09 ± 13.9 [a]	415.83 ± 23.0 [b]	378.47 ± 12.3 [a]	368.52 ± 20.4 [a]

Values are mean ± SD of 6 rats from each group
Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT)

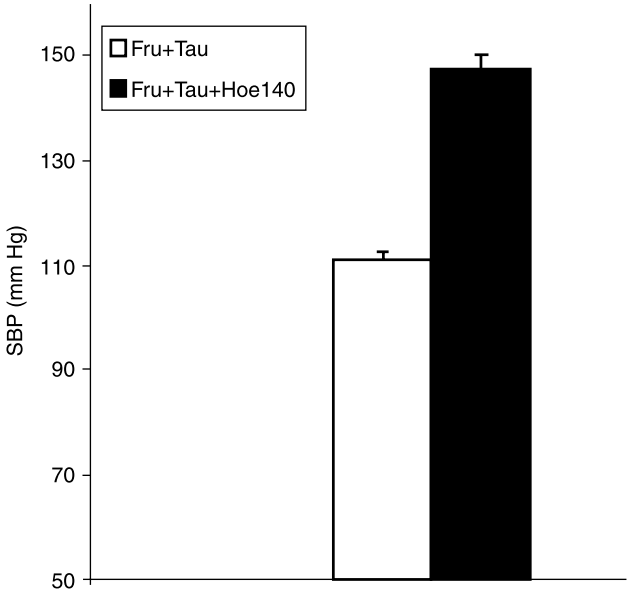

Fig. 1. Effect of taurine on systolic blood pressure (*SBP*) in fructose-fed hypertensive rats with or without simultaneous administration of Hoe 140 (100 μg/day/kg s.c.). Values are mean ± SD of 6 rats from each group

in control rats treated with taurine as compared to control rats. Heart weight: body weight ratio was significantly increased in fructose-fed rats as compared to control rats. The increased hypertrophy was reduced in taurine treated fructose-fed rats.

Co-administration of HOE-140 blocked the effects of taurine in fructose-fed rats (Fig. 1). This is evident from the higher blood pressure values in co-treated animals as compared to those treated only with taurine.

Table 3 gives the activity of kallikrein in different tissues and the concentration of sodium in plasma and urine of control and experimental animals. Kallikrein activity was significantly lower in heart, kidney, plasma and urine of fructose-fed rats ($p < 0.05$) as compared to control rats. Taurine treatment to the fructose-fed rats resulted in increased activity of kallikrein in plasma, tissues and in urine. No significant alternations were observed in the kallikrein activity in tissues and in plasma in CON-TAU as compared to CON. However increased excretion of kallikrein activity was observed in the CON-TAU as compared to CON.

Increased plasma concentration and reduced excretion of sodium in fructose-fed rats as compared to rats fed commercial diet is suggestive of sodium retention in these rats. The alterations in sodium homeostasis in fructose-fed rats are significantly modified by taurine. However no significant changes was observed in urinary excretion of sodium in CON-TAU group as compared to CON rats.

Table 3. Activity of kallikrein and levels of sodium in control and experimental animals

	CON	FRU	FRU-TAU	CON-TAU
Kallikrein				
Heart (Units/mg protein)	5.35 ± 0.23 [a]	4.79 ± 0.47 [b]	5.30 ± 0.09 [a]	5.44 ± 0.32 [a]
Kidney (Units/mg protein)	4.68 ± 0.43 [a]	2.66 ± 0.23 [b]	4.44 ± 0.13 [a]	4.92 ± 0.22 [a]
Plasma (U/L)	136.51 ± 1.36 [a]	89.64 ± 1.78 [b]	138.55 ± 5.33 [a]	138.34 ± 1.83 [a]
Urine (U/L)	138.2 ± 9.5 [a]	92.3 ± 10.2 [b]	178.1 ± 5.6 [c]	165.5 ± 11.5 [d]
Sodium				
Plasma (mequiv/L)	123.8 ± 8.37 [a]	138.6 ± 6.8 [b]	130.0 ± 6.8 [ac]	119.8 ± 4.8 [a]
Urine (mmol/day)	12.8 ± 0.72 [a]	9.3 ± 0.6 [b]	12.9 ± 0.6 [a]	13.1 ± 0.7 [a]

Values are mean ± SD of 6 rats from each group
Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT)

Discussion

The mean final body weights of the various groups of animals were not significantly different from each other. Rats treated with taurine in drinking water (FRU-TAU and CON-TAU groups) showed a significant increase in fluid intake. This could be due to the diuretic effect of taurine (Kohashi et al., 1990). Urine volumes of these animals (FRU-TAU and CON-TAU groups) were higher than those of the control animals, which could be in response to the increased fluid intake of these rats. There was no significant change in food intake between control and experimental animals.

The fact that fructose-fed rats develop hypertension was first reported by Hwang et al. (1989). The high blood pressure has been linked to the hyperinsulinemia produced in them. Hyperinsulinemia is associated with sodium retention by kidneys (De Fronzo, 1981) and with vascular wall hypertrophy resulting in hypertension (Baron et al., 1993). Reduced renal function as indicated by decreased creatinine clearance and sodium retention in fructose-fed rats has been reported earlier from this laboratory (Anuradha and Balakrishnan, 1999).

In the present study urinary kallikrein activity was lower in fructose-fed rats than in control rats. Kallikrein is excreted at the level of collecting tubules and is excreted less in hypertension (Vio et al., 1992). The major function of the renal kallikrein-kinin system is to excrete sodium and water when excess sodium is present. The decreased kinin production could result in natriuresis since kinins affect Na^+ and water excretion and autoregulation of kidney by releasing prostaglandins and nitric oxide (Carretero and Scicli, 1990).

Taurine was effective in reducing blood pressure and cardiac hypertrophy. Taurine has been shown to inhibit cardiac myocyte hypertrophy and fibroblast proliferation induced by angiotensin II treatment of primary cultures of rat heart (Takahashi et al., 1997). Taurine has been reported to reduce blood pressure in experimental hypertensive animal models (Meldrum et al., 1994) and in hypertensive humans (Fujita et al., 1987). In addition to taurine's ability to modulate cardiovascular function, taurine may have other actions to retard some of the adverse chronic effects of elevated blood pressure (Dawson et al., 2000).

The precise mechanism by which taurine controls blood pressure has not yet been elucidated. The effect is attributed to its antinatriuretic action (Okabayashi et al., 1984), which could be brought about by the activation of the renal kallikrein-kinin system. Kallikrein activity in kidney and other tissues were higher in taurine-treated rats in the present study.

In a previous study we found that taurine could improve insulin action in fructose-fed rats (Nandhini and Anuradha, 2002). Henrikson et al. (1998) reported that kinins could enhance insulin stimulated glucose transport activity in skeletal muscle of obese rats. Taken together it can be speculated that taurine could act through kinins. Infact taurine was shown to amplify renal kallikrein gene expression and to prevent salt-induced hypertension in Dahl-rats (Ideshi et al., 1994).

The involvement of kinins in the mechanism of action of taurine was further evidenced by inhibitor studies. Administration of HOE 140, which is a powerful tool for investigating the putative role of kinins, antagonized the effect of taurine and blocked the antihypertensive effect of taurine. The results of the study show that taurine supplementation lowers blood pressure by augmenting the production of kallikrein protein and kinin availability in this model. These findings necessitate further investigations owing to the functional importance of kinins.

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