Clinical and Experimental Pharmacology and Physiology (2006) **33**, 734–739

BEHAVIOUR OF THE ANTI-OXIDANT DEFENCE SYSTEM AND HEME OXYGENASE-1 PROTEIN EXPRESSION IN FRUCTOSE-HYPERTENSIVE RATS

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SUMMARY

1. Addition of fructose to a rat diet for various periods of time leads to hypertension, hyperinsulinaemia and dyslipidaemia and provides a model for testing oxidative stress parameters in the animals.

2. In the present study, oxidative stress generation, the soluble and enzymatic defence system and heme oxygenase-1 (HO-1) protein expression were investigated in the heart, liver and kidney of rats fed fructose for a period of 1 or 8 months.

3. Compared with the control group, fructose-hypertensive rats showed increased in lipid peroxidation only in the heart after both 1 and 8 months of fructose treatment. Changes in the behaviour of the soluble and enzymatic defence system and HO-1 protein expression were different depending on the organ. Increased or unaltered activities of anti-oxidant enzymes were found in the liver and kidney, respectively. Induction of HO-1 prevented the generation of oxidative stress in the liver, where the activity of anti-oxidant defence enzymes was not reduced. Increased expression of HO-1 protein was not able to prevent the generation of oxidative stress in the heart, where fructose treatment diminished the activity of anti-oxidant enzymes.

4. The results of the present study demonstrate that upregulation of HO-1 may prevent the generation of oxidative stress only when the anti-oxidant defence system is still operative.

Key words: anti-oxidant enzymes, fructose hypertension, heme oxygenase, oxidative stress, rats.

INTRODUCTION

Diets with a high fructose content have been used in animal models to induce the metabolic changes observed in syndrome X, a disorder in which insulin resistance, hypertension, dyslipidaemia and a high incidence of cardiovascular diseases occur.[1] Moreover, it is known that fructose feeding for 2 weeks induces left ventricular hypertrophy in rats.[2]

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Received 25 November 2005; revision 9 January 2006; accepted 30 January 2006.

It has been known for some time that increases in dietary carbohydrate intake can raise blood pressure in experimental animals. Increased fructose intake induces hypertension, as demonstrated in rats by Dai and McNeill.[3] They found a correlation between the hypertension induced and the concentration of fructose administered, as well as with the duration of fructose treatment. The addition of 10% fructose in the drinking water over a period of 7 or more days appeared to rapidly produce hypertension. The underlying mechanisms responsible in animal models for the detrimental consequences of a diet with a high fructose content are not clear, but it is possible that fructose feeding facilitates oxidative damage. This hypothesis is supported by studies demonstrating deleterious effects of fructose either when anti-oxidant defences decrease or when free radical production increases.[4] Reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide and hydroxyl radical, are implicated in the pathogenesis of hypertension and endothelial damage.[5] The generation of ROS is enhanced through different mechanisms, including xanthine oxidase activation, NADH auto-oxidation and inactivation of superoxide dismutase (SOD).[6] Superoxide anion produces endothelial dysfunction in diabetes[7] and has been implicated in the oxidative damage in smooth muscle and vascular endothelial cells.[8]

Different anti-oxidant enzymes protect cells from ROS production. For example, superoxide dismutase (SOD) not only serves to scavenge superoxide anion, but it also produces the more stable hydrogen peroxide and catalase (CAT) and glutathione peroxidase (GPX) are two of the most important scavengers of hydrogen peroxide, converting it into water.[9] An exaggerated ROS increase and/or a reduction in the activity of anti-oxidant enzymes determine oxidative damage in tissues.

Heme oxygenase (HO) is the rate-limiting enzyme in heme catabolism, which leads to the generation of CO, free iron and biliverdin. In most mammals, biliverdin is subsequently converted to bilirubin by biliverdin reductase.[10,11] Three mammalian HO isoforms have been identified, one of which, HO-1, is a stress-responsive protein induced by various oxidative agents.[12] In recent years, several groups have demonstrated that the expression of HO-1 is upregulated by its substrate heme, as well as by a variety of stress stimuli. These studies disclose an important cellular defence role for HO-1 against oxidant injury.[13]

Because as far as we know there have been no investigations testing the role of HO and the anti-oxidant defence system in fructose-fed animals, in the present study we examined the effect of fructose administration on the activity of anti-oxidant enzymes and the expression of HO-1 protein in the heart, liver and kidney of induced hypertensive rats.

METHODS

Animal treatments

Male Sprague-Dawley rats, with an average weight of 375 g, were used. Animals were housed in individual cages in a room maintained at 25 ± 2°C on a 12 h light/dark cycle and had free access to standard rat chow and tap water. Two groups of rats were supplemented with fructose (10% w/v) in the drinking water, one over a period of 1 month (F1) and the other over a period of 8 months (F8). Control groups without treatment for 1 (C1) and 8 months (C8) were also included in the study. Each group consisted of seven animals. Animals were treated following the guidelines established by the Animal Care and Use Committee of the Argentine Association of Specialists in Laboratory Animals (AADEALC) and were in accordance with the *Guide to the Care and Use of Experimental Animals* published by the Argentine Council on Animal Care (http://www.iclas.org).

Measurement of systolic blood pressure

Systolic blood pressure (SBP) was monitored indirectly in conscious, prewarmed, slightly restrained rats using the tail-cuff method and recorded on a Grass model 7 polygraph (Grass Instruments, Quincy, MA, USA).

Enzyme preparations and assays

Rats were decapitated and the kidneys, livers and hearts were excised, washed with an ice-cold saline solution (0.9% NaCl) and homogenized in a Potter-Elvehjem homogenizer (Thomas, New York, USA). The activity of SOD, CAT and GPX was determined spectrophotometrically in tissue homogenates prepared in a medium containing 140 mmol/L KCl and 25 mmol/L potassium phosphate buffer (pH 7.4) and centrifuged at 600 *g* for 10 min. The supernatant, a suspension of preserved organelles, was used as the homogenate. Catalase activity was determined by measuring the decrease in absorbance at 240 nm,[14] GPX activity was assayed by following NADPH oxidation at 340 nm, where one unit of the enzyme represents a decrease of 1 mmol NADPH/min under assay conditions;[15] and SOD activity was determined by inhibition of the formation of adrenochrome at 480 nm,[16] where one unit of SOD activity is defined as the amount of enzymatic protein required to inhibit 50% of adrenaline auto-oxidation.

Lipid peroxidation

Homogenate lipid peroxidation was determined by measuring the rate of production of thiobarbituric acid-reactive substances (TBARS; expressed as malondialdehyde (MDA) equivalents). One volume of homogenate was mixed with 0.5 volumes of trichloroacetic acid (15% w/v) and centrifuged at 2000 *g* for 10 min. The supernatant (1 mL) was mixed with 0.5 mL thiobarbituric acid (0.7% w/v) and boiled for 10 min. After cooling, sample absorbance was determined spectrophotometrically at 535 nm. The concentration of MDA was calculated using a ε-value of 1.56×10^5 L/mol per cm.[17]

Reduced glutathione

Reduced glutathione was determined as non-protein and total sulphydryl content in rat tissues using the method of Sedlak and Lindsay.[18] Briefly, tissues were homogenized in 5.0 mL cold KCl (1.15%) and proteins were precipitated with trichloroacetic acid. The reaction mixture contained 0.5 mL supernatant, 2.0 mL Tris-EDTA buffer (pH 8.9) and 0.1 mL of 6 mmol/L 5,5′-dithiobis 2-nitrobenzoic acid (DTNB). Absorbance was determined at 412 nm. Results are expressed as μmol GSH/mg protein.

Western blot analysis of HO-1 expression

Samples of homogenate were analysed for HO-1 using the western inmunoblot technique, as described previously.[19] For the HO assay, the homogenate was prepared using 4 volumes of ice-cold 0.25 mol/L sucrose solution containing 1 mmol/L phenylmethylsulphonyl fluoride (PMSF), 0.2 mmol/L EDTA and 50 mmol/L potassium phosphate buffer (pH 7.4). Homogenates were centrifuged at 20 000 *g* for 20 min and supernatant fractions were centrifuged at 150 000 *g* for 90 min. The microsomal pellet obtained was washed and resuspended in 20 mmol/L potassium phosphate buffer (pH 7.4), containing 135 mmol/L KCl, 1 mmol/L PMSF and 0.2 mmol/L EDTA, to a protein concentration of 10 mg/mL. Microsomal HO-1 was obtained by a similar procedure as described elsewhere.[10] Protein (50 μg) from the homogenates of control and treated rats was run on sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) using a 12% acrylamide resolving gel (Mini Protean II System; Bio-Rad, Hertz, UK). Separated proteins were transferred to nitrocellulose membranes and non-specific binding of antibodies was blocked with 3% non-fat dry milk in PBS, pH 7.4, for 1 h at room temperature. Membranes were then probed with polyclonal anti-HO-1 antibody (Stressgen Biotechnologies, San Diego, CA, USA; 1 : 300 dilution in Tris-buffered saline, pH 7.4) overnight at 4°C. Immune complexes were detected using a secondary antibody (1 : 1500) and were visualized using ECL reagent (Amersham Pharmacia, Buckinghamshire, UK). The intensity of the bands was analysed using Gel-Pro® analyser 3.1 version (Media Cybernetics, New York, USA).

Protein determination

Protein concentration was evaluated according to the method of Lowry *et al*.[20] using bovine serum albumin as the standard.

Biochemical determinations

After overnight fasting, blood samples for triglycerides and insulin determination were taken from all rats by the tail-bleeding method at the end of the experimental period. The concentration of plasma triglycerides was determined using commercial kits for enzymatic colourimetric methods (Wiener Laboratory, Rosario, Argentina).

Plasma insulin was assayed using an ELISA kit for insulin (Crystal Chem, Downers Grove, IL, USA).

Statistical analysis

Results are expressed as the mean±SEM. Data were analysed statistically by factorial analysis of variance (ANOVA) followed by the Neuman–Keuls' test for comparison of means. Values are reported as the mean±SEM of seven rats per group.

RESULTS

Systolic blood pressure measurement and biochemical determinations

Systolic blood pressure, insulin and plasma triglycerides are given in Table 1. Compared with their corresponding controls (C1), rats in the F1 group had increased SBP and higher blood pressure values were observed in the F8 group compared with the C8 rats.

Increased insulin plasma levels were detected in both F1 and F8 rats, whereas triglyceride levels were slightly and significantly increased only in F8 rats.

Thiobarbituric acid-reactive substances content

The formation of TBARS in organs in several pathologies is a reliable indicator of free radical production in tissues. As shown in Fig. 1a, a 20% increment in TBARS content was obtained in hearts of F1 rats and a marked increase (148%) in lipid peroxidation was observed in F8 rats compared with the corresponding control animals. No significant differences in TBARS content were found in liver and

Table 1 Effect of fructose treatment for 1 or 8 months on systolic blood pressure and plasma concentrations of insulin and triglycerides

Rat group	SBP (mmHg)	Insulin (ng/mL)	Triglycerides (mg/dL)
C1	114 ± 3	2.08 ± 0.20	0.91 ± 0.09
F1	129 ± 2***	4.95 ± 0.28**	0.92 ± 0.09
C8	117 ± 4	2.39 ± 0.26	0.92 ± 0.09
F8	143 ± 3***	20.19 ± 2.10***	1.12 ± 0.07*

Data are the mean±SEM of seven rats in each group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the corresponding control.

F1, F8, rats treated with fructose for 1 and 8 months, respectively; C1, C8, corresponding time controls; SBP, systolic blood pressure.

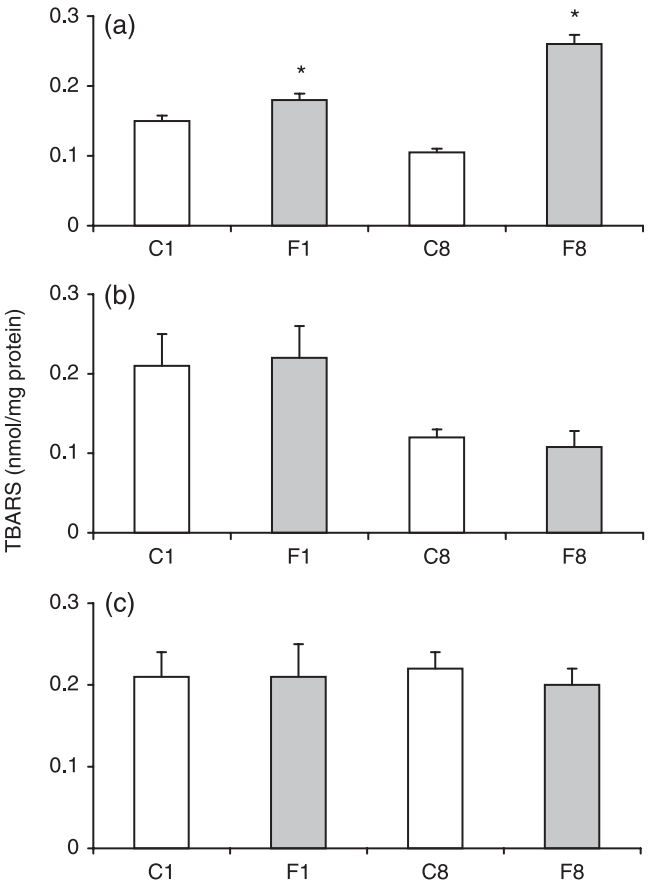

Fig. 1 Effect of fructose treatments on lipid peroxidation in the (a) heart, (b) liver and (c) kidney. F1, F8, rats treated with fructose for 1 and 8 months, respectively; C1, C8, corresponding time controls. Data are the mean±SEM of seven rats in each group. *$P < 0.001$ compared with respective controls. TBARS, thiobarbituric acid-reactive substances.

kidney homogenates from F1 and F8 rats compared with controls animals (Fig. 1b,c).

Glutathione content

Reduced glutathione is the major low molecular weight hydrosoluble thiol anti-oxidant inside cells. Figure 2a shows that, compared with the respective controls, fructose administration did not modify heart GSH levels in the F1 group, but increased GSH by approximately 42% in the F8 group. In the liver, compared with the respective

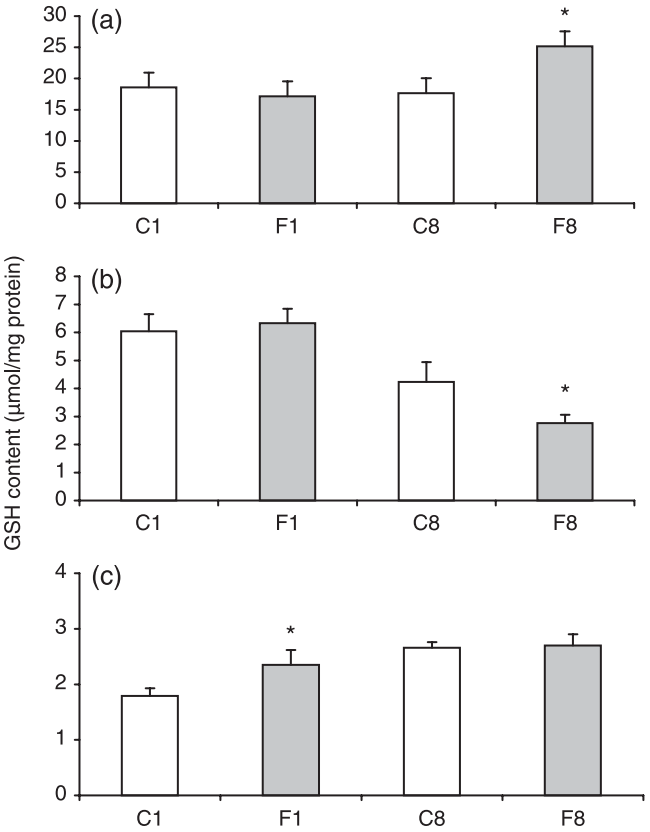

Fig. 2 Effect of fructose treatments on reduced glutathione (GSH) content in the (a) heart, (b) liver and (c) kidney. F1, F8, rats treated with fructose for 1 and 8 months, respectively; C1, C8, corresponding time controls. Data are the mean±SEM of seven rats in each group. *$P < 0.001$ compared with respective controls. TBARS, thiobarbituric acid-reactive substances.

controls, GSH content was unaltered in the F1 group, but a 33% decrease was found in F8 animals (Fig. 2b). In contrast, in the kidney, a 30% increase over GSH levels in C1 rats was observed in the F1 group, whereas GSH levels in the F8 group remained similar to those of the C8 controls (Fig. 3c).

Anti-oxidant enzyme activities

The activity of SOD and GPX increased in the heart by approximately 50% compared with controls after 1 month of fructose treatment (Table 2). However, in rats treated with fructose for 8 months, the activity of these enzymes decreased by approximately 40% with respect to control levels. Catalase activity decreased in both the F1 and F8 groups (32% and 50%, respectively) compared with the respective controls.

Table 3 shows that, in liver of the F1 group, SOD activity was increased 21% over the control group, whereas after 8 months of fructose treatment SOD activity had decreased by 42% with respect to controls. Catalase activity was unaltered in the F1 group, whereas a 29% increase in CAT activity was observed in the F8 group compared with control values. In addition, GPX enzyme activity increased approximately 65% and 25% in the F1 and F8 groups, respectively, compared with the C1 and C8 animals.

In the kidney, after 1 month of fructose treatment, SOD and GPX activity had increased 20% and decreased 19%, respectively,

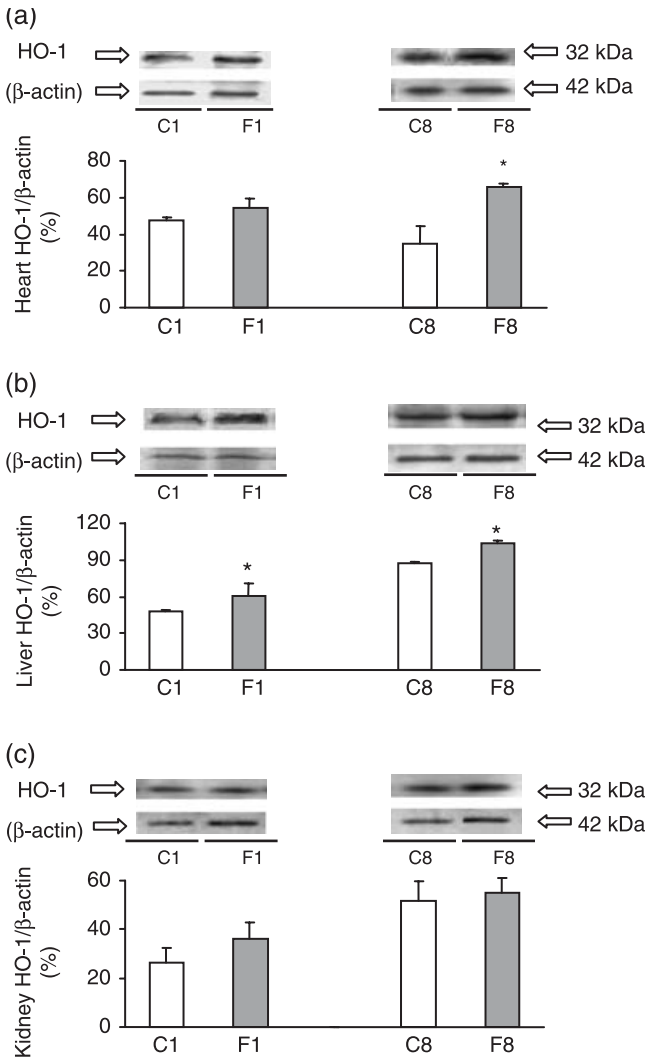

Fig. 3 Western blot analysis of heme oxygenase-1 (HO-1) expression in the (a) heart, (b) liver and (c) kidney. F1, F8, rats treated with fructose for 1 and 8 months, respectively; C1, C8, corresponding time controls. Densitometry was performed to quantify HO-1 protein expression. Blots shown are representative of three blots with a total of four to five samples/group between the three blots. $*P < 0.05$ compared with control.

compared with the control group, whereas there was no change in CAT activity. No changes in kidney anti-oxidant enzyme activities were detected after 8 months of fructose treatment (Table 4).

Western blot analysis of HO

In the heart, HO-1 expression was significantly increased (90%) only in the F8 group compared with C8 rats (Fig. 3a). In the liver, HO-1 protein expression was increased by 50% and 25% in the F1 and F8 groups, respectively (Fig. 3b). However, no significant difference in kidney HO-1 expression was observed in either the F1 or F8 groups compared with control animals (Fig. 3c).

DISCUSSION

Anti-oxidant/oxidant balance is well established as an important physiological regulator of arterial pressure and, recently, its role

Table 2 Effect of fructose treatment for 1 or 8 months on the activity of anti-oxidant enzymes in the heart

Rat group	SOD (U/mg protein)†	CAT (pmol/mg protein)	GPx (U/mg protein)‡
C1	4.16 ± 0.47	0.151 ± 0.005	0.54 ± 0.05
F1	6.20 ± 0.65***	0.102 ± 0.006***	0.81 ± 0.03***
C8	5.00 ± 0.50	0.085 ± 0.005	0.35 ± 0.01
F8	3.00 ± 0.30**	0.043 ± 0.001*	0.21 ± 0.03*

Data are the mean±SEM of seven rats in each group. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared with the corresponding control.

Enzymatic activities were assayed as described in the Methods.

†One unit of superoxide dismutase (SOD) activity is defined as the amount of enzyme required to inhibit adrenaline auto-oxidation by 50%.

‡One unit of the enzyme represents a decrease of 1 mmol NADPH/min under the assay conditions.

F1, F8, rats treated with fructose for 1 and 8 months, respectively; C1, C8, corresponding time controls; CAT, catalase; GPx, glutathione peroxidase.

Table 3 Effect of fructose treatment for 1 or 8 months on the activity of anti-oxidant enzymes in the liver

Rat group	SOD (U/mg protein)†	CAT (pmol/mg protein)	GPx (U/mg protein)‡
C1	10.52 ± 1.01	3.36 ± 0.37	0.33 ± 0.03
F1	12.83 ± 2.02**	3.52 ± 0.40	0.54 ± 0.06*
C8	9.00 ± 0.90	2.70 ± 0.30	0.28 ± 0.01
F8	5.30 ± 0.45*	3.50 ± 0.30**	0.35 ± 0.03**

Data are the mean±SEM of seven rats in each group. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared with the corresponding control.

Enzymatic activities were assayed as described in the Methods.

†One unit of superoxide dismutase (SOD) activity is defined as the amount of enzyme required to inhibit adrenaline auto-oxidation by 50%.

‡One unit of the enzyme represents a decrease of 1 mmol NADPH/min under the assay conditions.

F1, F8, rats treated with fructose for 1 and 8 months, respectively; C1, C8, corresponding time controls; CAT, catalase; GPx, glutathione peroxidase.

Table 4 Effect of fructose treatment for 1 or 8 months on the activity of anti-oxidant enzymes in the kidney

Rat group	SOD (U/mg protein)†	CAT (pmol/mg protein)	GPx (U/mg protein)‡
C1	4.00 ± 0.05	1.20 ± 0.10	0.49 ± 0.02
F1	4.80 ± 0.30**	1.44 ± 0.05	0.40 ± 0.03**
C8	4.20 ± 0.40	1.12 ± 0.10	0.54 ± 0.04
F8	4.10 ± 0.03	0.98 ± 0.10	0.54 ± 0.05

Data are the mean±SEM of seven rats in each group. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared with the corresponding control.

Enzymatic activities were assayed as described in the Methods.

†One unit of superoxide dismutase (SOD) activity is defined as the amount of enzyme required to inhibit adrenaline auto-oxidation by 50%.

‡One unit of the enzyme represents a decrease of 1 mmol NADPH/min under the assay conditions.

F1, F8, rats treated with fructose for 1 and 8 months, respectively; C1, C8, corresponding time controls; CAT, catalase; GPx, glutathione peroxidase.

in the pathogenesis of various forms of hypertension has been substantiated.[21–24]

Different studies performed in rats have shown changes in the oxidative status due to the damage caused by high fructose consumption under pro-oxidant conditions.[4] The hypothesis that fructose itself could be pro-oxidant has recently been suggested, but the exact mechanisms are still unclear.[4] Moreover, several studies have provided evidence suggesting that enhanced ROS production deregulates physiological processes implicated in the pathogenesis of arterial hypertension.

The present study demonstrated that the maximum increase in SBP following fructose treatment was 26 mmHg. Thus, it is apparent that fructose-induced hypertension in rats is a relatively mild hypertension compared with the genetic hypertension in spontaneously hypertensive rats (SHR) and the renovascular or deoxycorticosterone acetate (DOCA)-induced hypertension in rats. The present results indicated that fructose administration slightly and significantly increased plasma triglycerides in the F8 group only. It is interesting to note that the effect of fructose on insulin plasma levels was greater in F8 rats than in F1 rats. The present study indicates that the hyperinsulinaemia developed by F8 rats is related to the increment of SBP in those animals, in agreement with the results of Verma *et al*.[25] Although hyperinsulinaemia has been proposed to increase SBP through several mechanisms, including activation of the sympathetic nervous system, sodium retention and vascular smooth muscle hypertrophy, the precise nature of these mechanisms remains elusive.[25]

The levels of anti-oxidant enzymes are sensitive to oxidative stress and increased or decreased levels have been reported in hypertension, as well as in various pathologies. It has been shown that the expression of anti-oxidant defence enzymes is enhanced in the myocardium from SHR following the induction of oxidative stress.[26] In contrast, the activity of anti-oxidant enzymes may be decreased, mainly when the oxidative stress load overcomes the defence potential.[26]

The regulation of anti-oxidant enzymes in tissues of higher animals depends on diverse factors, including organ specificity, age, prevailing hormone profile and the availability of active site cofactors, which have the potential to limit the expression of enzyme activity.[27]

In addition, there is a wide range of non-enzymatic and enzymatic factors involved in the defence mechanism against oxidative stress; in the present study we have chosen only some relevant soluble (GSH) and enzymatic defence systems (SOD, CAT and GPX) as representatives of the generation of oxidative stress, similar to our previous work.[12]

In heart tissue of rats fed fructose for 1 month, the increase in SOD and GPX activities was insufficient to prevent the increase in TBARS levels. In addition, GSH content and HO-1 protein expression remained similar to control values and a decrease in CAT activity was observed. The increase in SOD activity, as well as the reduced CAT activity, contributes to a higher production of H_2O_2, which could be responsible for the generation of oxidative stress. In F8 animals, the considerable increase in TBARS content was probably due to a general decrease in anti-oxidant enzyme activity, despite the fact that the GSH content was significantly enhanced. A possible explanation for the decrease in anti-oxidant enzymes in F8 rats could be the significant enhancement in plasma insulin concentrations (a fourfold increase compared with F1 rats). This possibility is supported by results reported by Somogyi *et al*.,[28] who demonstrated that insulin treatment decreased the anti-oxidant defence mechanism in experimental diabetes. The morbidity and mortality associated with diabetes is the result of the myriad complications related to the disease. One of the most explored hypotheses to explain the onset of complications is a hyperglycaemia-induced increase in oxidative stress and, once formed, ROS deplete anti-oxidant defences, rendering the affected cells and tissues more susceptible to oxidative damage.[29] It has been reported that the anti-oxidant enzymes SOD, CAT and GPX are significantly altered (increased or decreased) in different types of diabetic patients,[30–32] whereas TBARS[28,30–32] and triglycerides[28,30,31] are significantly increased. In addition, hyperglycaemia-induced oxidative stress is a risk factor for cardiovascular disease.[28] Hyperlipidaemia also produces oxidative stress in humans.[33]

A marked increase in HO-1 expression was found in the hearts of F8 rats, but this increment was not enough to avoid the generation of oxidative stress. These results indicate tha, after short fructose treatment (1 month), oxidative stress occurred and this situation was aggravated after fructose treatment over a period of 8 months.

In the liver, except for CAT activity, which, in F1 animals, was similar to control values, increased SOD and GPX activities in addition to the unaltered GSH content and increased HO-1 expression were apparently sufficient to cope with the generation of oxidative stress, at least in our experimental design. Similarly, in F8 rats, despite the diminished GSH content and SOD activity, the enhanced CAT and GPX activities and HO-1 expression were enough to prevent the increase in TBARS content.

In the present study, the kidney appears to be the organ less affected by fructose treatment and only a slight increase in GSH content and SOD activity was found in the liver of F1 rats. In addition, a slight and significant decrease in GPX activity was observed. In F8 rats, all parameters measuring the generation of oxidative stress were unaltered and HO-1 expression was no different to that in controls in both F1 and F8 rats.

A diet rich in fructose induces hyperinsulinaemia, hypertriglyceridaemia, hypertension, renal changes and left ventricular hypertrophy.[34] Cosenzi *et al*.[35] have demonstrated that, after 4 weeks of a fructose-rich diet, rats had enlarged kidneys, the size of the glomeruli was increased and there was deposition of collagen and fibronectin. Left ventricular hypertrophy, characterized by an increased size of the myocytes and interstitial deposition of collagen, has also been observed in rats on a high-fructose diet.[35] Despite the fact that a high-fructose diet in rats is accompanied by renal and cardiac damage, the oxidative balance in both organs was significantly dissimilar and no oxidative stress was observed in the kidney although it was found in rat heart. Instead, in the present study, the liver, which is not a target organ in the fructose-fed hypertension model, showed the most favourable enzymatic defence mechanism against oxidative damage.

Several studies suggest that oxidant species play a major role in the induction of HO either directly or by GSH depletion.[36–41] In the present study, we observed that, in the kidney, there is no generation of oxidative stress after either short- or long-term fructose administration and that HO-1 protein expression in fructose-fed rats was similar to that in control rats. In the liver, the enhancement in some anti-oxidant defence parameters, together with an increase in HO-1 protein expression, prevent the generation of oxidative stress in either F1 or F8 rats. However, in the heart, the increase in SOD and GPX activity, together with unaltered HO-1 protein expression, was not sufficient to avoid the generation of oxidative stress in F1 animals. In addition, long-term fructose treatment aggravated oxidative damage and, therefore, the increase in HO-1 levels was not able to prevent oxidative stress injury.

In conclusion, the administration of fructose to rats produced different changes in the activity of anti-oxidant enzymes and lipid peroxidation depending on the organ. In the liver and kidney, evaluated after 1 or 8 months fructose treatment, we found an adaptive response to oxidative stress. The heart was the tissue most affected by ROS production and, for both periods of fructose administration, the oxidative damage load overcame the defence potential in the heart. These data clearly demonstrate that upregulation of HO-1 only is capable of prevent the generation of oxidative stress when the anti-oxidant defence system remains operative.

ACKNOWLEDGEMENTS

This work was supported by grants from Universidad de Buenos Aires (Argentina) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) (Argentina). CP and MLT are career investigators from CONICET.

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