Potential Role of Glycerol Leading to Rat Fructose Hypertension

Pablo F. Damiano, María I. Rosón, Inés Armando, Susana Nowicki, Eduardo Dascal, Luis Cuniberti, Liliana E. Albornoz, Ignacio J. de la Riva

Abstract—A fructose-enriched diet promotes hypertension in rats. We thought that an enhancement of the glycolytic and/or lipid disorder(s) that raise blood pressure could be the cause. Therefore, we studied 4 groups of Sprague-Dawley rats (±200 g): (1) control rats received a standard diet and tap water; (2) the glycerol group of rats received a standard diet and 0.54 mol/L glycerol in tap water; (3) the fructose group was given a fructose-enhanced diet (chow had 55% fructose instead of dextrin) and tap water; and (4) the fructose-glycerol group was given the fructose-enhanced diet and 0.54 mol/L glycerol in drinking water. At the end of the second week, the findings were as follows. Blood pressure was 149±2 mm Hg in the fructose-glycerol group versus 129±2 (P<0.001), 131±2 (P<0.001), and 140±3 (P<0.005) mm Hg in the control, glycerol, and fructose groups, respectively. Insulinemia was higher in the fructose-glycerol group than the control (P<0.001), glycerol (P<0.001), and fructose groups (P<0.001); triglyceridemia was higher in the fructose-glycerol (P<0.02), fructose (P<0.05), and glycerol groups (P<0.02) than the control group. Thoracic aorta rings showed a lower ED50 to 12,13-phorbol dibutyrate in the fructose-glycerol group than in the control (P<0.001), glycerol (P<0.002), and fructose groups (P<0.001). In conclusion, glycerol-fructose administration resulted in hypertriglyceridemia, hyperinsulinemia, and increased vascular sensitivity to 12,13-phorbol dibutyrate (with respect to the control group), and significantly greater expression of protein kinase C α and βII (with respect to the glycerol group). (Hypertension. 1999;34[part 2]:1007-1011.)

Key Words: fructose • hypertension • insulin • glycerol • triglycerides

In experimental animals, fructose overload can raise blood pressure (BP) and cause hypertriglyceridemia, hyperinsulinemia, impaired glucose tolerance, and insulin resistance in several target tissues.1–5 In this regard, many investigators have suggested that increased insulin levels and/or metabolic responses may be causally related to elevated BP.6–7 This possibility is supported by reports correlating hyperinsulinemia with hypertension.1,8 However, hyperinsulinemia and hypertension have been described as independent factors by other authors.7,9 Consequently, the underlying mechanisms of these 2 conditions are, as yet, undefined.

The lipid dysmetabolism that accompanies fructose overload is another possible cause of hyperinsulinemia and hypertension. In fact, a vicious cycle between hypertriglyceridemia and insulin resistance exists.10 Pretki and Corkey11 studied type II diabetes mellitus and proposed that hyperinsulinemia could be explained by alterations in the glycolytic pathway and lipid metabolism (hypertriglycerideridemia is one of the features of a lipid disorder). This metabolic abnormality is followed by an increase of long-chain acyl coenzyme A, which results in an elevation of insulin secretion. The hypertriglycerideridemia associated with fructose overload seems to result from lower plasma extrahepatic triglyceride lipase activity and greater VLDL-triglyceride secretion rates.12 Similarly, increased levels of glycerol in the diet induce hypertriglycerideridemia in the rat as a result of lower triglyceride clearance after decreased lipoprotein lipase activity.13 Lee et al4 found that raised levels of glucose increase diacylglycerol, which in turn activates protein kinase C (PKC) in the cultured vascular cells and in the aorta, heart, and other tissues from streptozotocin-diabetic rats15–17; PKC can also modulate contractions of vascular smooth-muscle cells.18,19 The liver and kidneys, which are formally recognized as the main organs responsible for glycerol metabolism (which leads to glycogenesis), account for less than 50% of endogenous glycerol clearance.20 This finding indicates that other tissues (putatively including the vascular tissue) that contain glycerol kinase in low concentrations may participate in its metabolism and, thus, potentiate diacylglycerol synthesis.

Accordingly, the objective of this study was to observe the effect of glycerol supplementation on BP to determine to...
what extent lipid and/or glycolytic pathways are primarily involved in fructose-caused hypertension.

**Methods**

**Animals**
Male Sprague-Dawley rats (Animal Facilities, Biochemistry College, Buenos Aires University) that weighed 180 to 230 g at the start of the study were used. Animals were maintained in a room at 22 ± 2°C, and the air was adequately recycled. Initially, all rats were fed a commercial standard laboratory chow (Asociación Cooperativas Argentinas) with the following composition (wt/wt): 20% proteins, 3% fat, 2% fiber, 6% minerals, and 69% starch and vitamin supplements. After 2 weeks, animals were identified by ear marks and randomly divided into 4 groups: (1) the control group continued on the standard diet and was given tap water to drink; (2) the glycerol group received the same solid diet and a glycerol solution (0.54 mol/L) was added to the tap water; (3) the fructose group received a special diet containing 14% starch and 55% fructose, instead of the 69% starch of the standard diet, and tap water to drink; and (4) the fructose-glycerol group received the same diet as group 3 and the 0.54 mol/L glycerol solution to drink. All groups were examined after 14 days.

**BP and Body Weight Measurement**
Rats were acclimated to the procedure of BP measurement at 1.00 PM twice a week starting 1 week before dietary manipulation and continuing through the experimental period. Indirect systolic BP was determined as previously reported. The mean of 3 consecutive stable readings was used as the measurement of the systolic BP of each rat for that day, and the average BP of the last 2 readings (11th and 13th days of the study) were used for statistical comparisons. The procedure for analyzing the BP data was similar to that reported by Hwang et al.1 In addition, rats were weighed before dietary manipulation and at the end of the study.

**Plasma Assays**
Animals fasted for 5 hours and then were anesthetized with an intraperitoneal injection of pentobarbital (60 mg/kg body weight); 90 minutes later, blood samples were drawn from the retro-ocular plexus. The samples were immediately centrifuged and frozen at −20°C until assayed for glucose, triglycerides, and insulin. Glucose (Kit Winner Gliceremia HK, UV) and triglyceride (Boehringer Mannheim GPO-PAP, enzymatic method kit) levels were measured by spectrophotometric methods (Automatic Analyzer, Abbott Spectrum CCX). Insulin was determined in plasma samples by radioimmunoassay using the method of Herbert et al.22

**Catecholamines in the Artery Wall**
The catechols in tissue homogenates from the abdominal aorta were determined by high-pressure liquid chromatography with electrochemical detection, as reported previously.23

**PKC Western Blots in the Artery Wall**
Descendent thoracic rat aorta segments were removed and homogenized in 0.3 mL of ice-cold homogenization buffer. PKCa, βII, and ε were determined by immunoblotting, as previously described.24 One sample from each of the 4 rat groups was processed in parallel in each gel. The autoradiograms were quantified by densitometric scanning. Values from the treated animals were expressed as percentages of a single control value.

**Contractility of Aorta Rings**
Batches of 4 rats (1 from each group) were killed daily by decapitation. The thoracic aorta was harvested, placed into cold Krebs solution, and prepared for contractility recording, as previously reported.21 Thereafter, the effect of 10−5 mol/L nitroprusside on baseline ring tension was observed. In other groups of rings, the dose response to 12,13-phorbol dibutyrate (PDBu; 5 · 10−8 to 5 · 10−6 mol/L) was determined.

**Statistical Analysis**
Results are expressed as mean ± SEM, and the significance level was P<0.05. Comparisons of data between different groups were made by 1-way ANOVA followed by a Newman-Keuls post hoc test when significance was indicated.

**Glycemia, Triglyceridemia, and Insulinemia**

**Figure 1.** BP in control (C), glycerol (G), fructose (F), and fructose-glycerol (FG) groups. *‘‘Groups against which significantly different values were found.

**Figure 2.** Glycemia, triglyceridemia, and insulinemia in control (C), glycerol (G), fructose (F), and fructose-glycerol (FG) groups; *Group against which significantly different values were found.
Results

Effect of Fructose and Glycerol on Arterial Pressure

After 14 days of experimental intervention, BP was as follows (mm Hg): control, 129±6; glycerol, 131±6; fructose, 140±3; and fructose-glycerol, 149±2. Significant differences were observed for the fructose group versus control (P<0.005) and glycerol groups (P<0.005) and for the fructose-glycerol group versus control (P<0.001), glycerol (P<0.001), and fructose groups (P<0.005) (Figure 1).

Fasting Glycemia, Triglyceridemia, and Insulinemia

On day 14, glycemia values for the glycerol, fructose, and fructose-glycerol groups were not significantly different from the control group. However, the glycemia concentration was significantly lower in the fructose-glycerol rats when compared with the fructose rats (P<0.05). Plasma triglyceride concentrations increased significantly with respect to the control group in all treated groups. Plasma insulin concentrations were significantly different from all other groups only in fructose-glycerol rats (Figure 1).

Vascular Contractility

No change in basal tone was discovered by the administration of 10⁻⁵ mol/L nitroprusside. However, dose-response curves with PDBu showed a significantly lower ED₅₀ (higher sensitivity) in aorta rings from the fructose-glycerol group with respect to those from the control (P<0.001), glycerol (P<0.002), and fructose (P<0.001) groups. Maximum tension was significantly higher only in the fructose group (Figure 3).

Catecholamines in the Artery Wall

No significant differences were observed among groups; nevertheless, with the exception of dopamine in the fructose group, all mean values were lower than in the control group (Table).

PKC Western Blot in Artery Wall

PKCe expression was significantly lower in the glycerol group than the control and fructose-glycerol groups, and PKCβII expression was significantly lower in the glycerol group than the fructose-glycerol group. No significant changes in PKCe were detected between groups (Figure 4).

Discussion

Although diabetes has been thoroughly investigated at the clinical and experimental levels, little attention has been paid to prior metabolic disorders and hypertension, which may ensue from excessive carbohydrate intake. The experimental model studied here is currently under intensive research; it mimics the risk factors of sweet-toothed subjects in the

<table>
<thead>
<tr>
<th>Catecholamines and DHPG in Homogenates of the Thoracic Aorta</th>
<th>Group</th>
<th>n</th>
<th>DHPG</th>
<th>Norepinephrine</th>
<th>Epinephrine</th>
<th>DOPA</th>
<th>Dopamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>96±28</td>
<td>80±9</td>
<td>13±3</td>
<td>29±6</td>
<td>7±2</td>
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<tr>
<td>Glycerol</td>
<td>10</td>
<td>51±7</td>
<td>75±11</td>
<td>6±1</td>
<td>19±5</td>
<td>4±1</td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>10</td>
<td>80±29</td>
<td>64±6</td>
<td>10±3</td>
<td>50±15</td>
<td>10±4</td>
<td></td>
</tr>
<tr>
<td>Fructose-glycerol</td>
<td>10</td>
<td>39±9</td>
<td>52±8</td>
<td>7±2</td>
<td>24±6</td>
<td>4±1</td>
<td></td>
</tr>
</tbody>
</table>

Values are given in pg of catecholamines or DHPG per mg of wet tissue and expressed as mean±SEM.

DOPA indicates 3,4-dihydroxyphenylalanine; and DHPG, 3,4-dihydroxyphenylglycol.
general population, ranging from “resistant” to “prediabetic” individuals.

Cardiac output is reportedly within normal limits in the fructose-overload experimental model; thus, peripheral vascular resistance must be increased to account for a high BP. Therefore, in addition to general metabolic disorders, this article examines some factors concerning the target organ, ie, the vascular wall. Glycerol supplementation was done to enhance the metabolic disorders leading to hypertension.

Peral de Bruno et al reported that aorta rings from rats after 10 weeks of a fructose diet have an increased basal tone, as shown by the presence of a relaxing effect to \(10^{-5}\) mol/L nitroprusside. In contrast, the present experiments failed to disclose changes in basal tone by nitroprusside in vitro after 2 weeks of fructose overload. Sensitivity to PDBu stimulation was significantly greater in vessels from our fructose-glycerol rats (Figure 3), although at a concentration of \(7.27 \times 10^{-7}\) mol/L, both the glycerol and fructose groups showed significantly greater vascular responses than controls. Such findings agree with the raised BP (putatively in peripheral resistance) that developed after 2 weeks both in the fructose and fructose-glycerol groups. Moreover, results from the fructose-glycerol group in particular support the potential effect of glycerol; BP, insulinemia, and vascular sensitivity to PDBu were all significantly greater when fructose was supplemented with 0.54 mol/L glycerol in drinking water.

The maximal tension to PDBu that developed was lower in the fructose-glycerol and glycerol groups and higher in the fructose group when compared with controls. This prompted us to investigate whether the different maximal responses to the direct PKC activator PDBu correlated with a different expression of PKC in the thoracic aorta within the studied groups. However, only in the glycerol group did a direct relationship between changes in the maximal PDBu-induced response and PKC expression exist.

Concerning catecholamines, an increased systemic sympathetic tone has been reported in fructose-overloaded rats. In this regard, although not significantly different among groups, all mean values in the aorta wall were lower in glycerol-supplemented groups (Table); these results render the increase in local catecholamine content in these groups unlikely. This suggestion is of particular interest for the fructose-glycerol rats in view of their greater incidence of hypertension.

In conclusion, our results showed the following: (1) oral glycerol administration per se (glycerol group) was accompanied by hypertriglyceridemia (as high as in other groups), normal insulinemia, and decreased thoracic aorta PKC \(\alpha\) expression; (2) in the fructose group, hypertriglyceridemia was again present, but rats failed to show hyperinsulinemia; (3) when glycerol was administered with fructose (fructose-glycerol group), hypertriglyceridemia, hyperinsulinemia, increased vascular sensitivity to PDBu, and significantly greater values of PKC\(\alpha\) and \(\beta\) expression with respect to the glycerol group were simultaneously present and accompanied by greater BP values with respect to the other 3 experimental groups. However, the mechanism linking glycerol potentiation to fructose overload remains unclear.

Acknowledgments

This work was supported by grants from the National Research Council (Consejo Nacional de Investigaciones Cientificas y Tecnicas [CONICET]) and the University of Buenos Aires, Argentina. Drs Armando, PhD, Norwicki, PhD, and de la Riva, PhD, are senior investigators from CONICET, Argentina.

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Hypertension. 1999;34:1007-1011
doi: 10.1161/01.HYP.34.4.1007

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