Fructose Stimulates Na/H Exchange Activity and Sensitizes the Proximal Tubule to Angiotensin II

Pablo D. Cabral, Nancy J. Hong, Md. Abdul Hye Khan, Pablo A. Ortiz, William H. Beierwaltes, John D. Imig, Jeffrey L. Garvin

Abstract—The proximal nephron reabsorbs 60% to 70% of the fluid and sodium and most of the filtered bicarbonate via Na/H exchanger 3. Enhanced proximal nephron transport is implicated in hypertension. Our findings show that a fructose-enriched diet causes salt sensitivity. We hypothesized that fructose stimulates luminal Na/H exchange activity and sensitizes the proximal tubule to angiotensin II. Na/H exchange was measured in rat proximal tubules as the rate of intracellular pH (pHi) recovery in fluorescent units/s. Replacing 5 mmol/L glucose with 5 mmol/L fructose increased the rate of pHi recovery (1.8±0.6 fluorescent units/s; P<0.02; n=8). Staurosporine, a protein kinase C inhibitor, blocked this effect. We studied whether this effect was because of the addition of fructose or removal of glucose. The basal rate of pHi recovery was first tested in the presence of a 0.6-mmol/L glucose and 1, 3, or 5 mmol/L fructose added in a second period. The rate of pHi recovery did not change with 1 mmol/L but it increased with 3 and 5 mmol/L of fructose. Adding 5 mmol/L glucose caused no change. Removal of luminal sodium blocked pHi recovery. With 5.5 mmol/L glucose, angiotensin II (1 pmol/L) did not affect the rate of pHi recovery (change, –1.1±0.5 fluorescent units/s; n=9) but it increased the rate of pHi recovery with 0.6 mmol/L glucose/5 mmol/L fructose (change, 4.0±2.2 fluorescent units/s; P<0.02; n=6). We conclude that fructose stimulates Na/H exchange activity and sensitizes the proximal tubule to angiotensin II. This mechanism is likely dependent on protein kinase C. These results may partially explain the mechanism by which a fructose diet induces hypertension.  (Hypertension. 2014;63:e68-e73.) ● Online Data Supplement

Key Words: angiotensin II  hypertension  sodium-hydrogen exchanger

Consumption of high-fructose corn syrup as a sweetener has increased dramatically in the past 3 decades. Consuming large quantities of fructose has been implicated in the epidemic of diabetes mellitus,1 obesity,2 renal failure,3,4 and hypertension.5 Most of the detrimental effects of fructose have been attributed to its chronic consumption.5,6 Chronically, a fructose-enriched diet modifies metabolic parameters, such as insulin sensitivity and uric acid production.6,7 These modifications have been suggested to be involved in the development of hypertension by enhancing sodium reabsorption in both the kidney and the intestine8,9 and thereby contributing to the development of hypertension in animals fed a fructose-enriched diet.10 All of these studies have demonstrated clearly the relationship between fructose consumption and development of hypertension. However, the intrinsic mechanisms at the tissue level are still not known.

The proximal nephron reabsorbs 60% to 70% of the sodium and water filtered by the glomerulus playing a pivotal role in the maintenance of extracellular fluid volume and therefore blood pressure regulation.11,12 It is also involved in reabsorbing most of the filtered bicarbonate via sodium/hydrogen exchanger 3 (NHE3).13 Enhanced sodium and fluid absorption by this nephron segment has been linked to hypertension in humans and several animal models.14,15

Angiotensin II is a key regulator of blood pressure through its action in the vasculature and the nephron.16,17 The exacerbated actions of the renin–angiotensin system have been implicated in many models of hypertension.18,19 It is also largely known that angiotensin II directly affects sodium and fluid transport in different segments of the nephron including the proximal tubule.20 Enhanced action of angiotensin II on the proximal tubule leads to salt-sensitive hypertension.19 Targeting the angiotensin II system in fructose-fed animals lowers blood pressure.21 Although these studies directly associated the angiotensin II system with the development of hypertension in fructose-fed animals, there is a lack of information on the possible mechanisms that associate angiotensin II to fructose-induced hypertension. We hypothesized that fructose, but not glucose, stimulates NHE activity and enhances the ability of angiotensin II to activate NHE in the proximal tubule.

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Methods

An expanded Methods section is available in the online-only Data Supplement.

Animal

All protocols and procedures were approved by the Institutional Animal Care and Use Committees of Case Western Reserve University and the Medical College of Wisconsin in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Results

We first studied the effects of 20% fructose in the diet on systolic blood pressure in animals fed either a normal or a high-salt diet. At baseline, the systolic blood pressure was similar between the experimental groups averaging 118±2 and 118±9 mm Hg. Fructose (20%) feeding for 7 days did not change systolic blood pressure significantly. Adding high salt to the diet caused systolic blood pressure to increase, and by day 7 of high-salt treatment the blood pressure of these rats reached a peak averaging 132±6 mm Hg compared with 119±2 mm Hg in the rats received 20% fructose alone (P<0.05; Figure 1).

Because the proximal nephron reabsorbs 60% to 70% of the sodium, water, and most of the filtered bicarbonate via NHE3, we tested the effects of replacing 5 mmol/L of glucose with 5 mmol/L of fructose on the rate of pHi recovery in isolated and perfused rat proximal tubules. In the presence of 0.6 mmol/L glucose+5 mmol/L fructose, the rate of pHi recovery increased 1.8±0.6 fluorescent units/s (FU/s; P<0.02; n=8) when compared with a basal pHi recovery rate in the presence of 5.5 mmol/L glucose. NHE3 and the Na/K-ATPase, the transporters mostly responsible for fluid and NaHCO3 absorption in the proximal tubule, are activated by protein kinase C (PKC).22 To study whether PKC was involved in the effects of fructose, we repeated the previous protocol in the presence of the general PKC inhibitor staurosporine (1 nmol/L). Under these circumstances, 0.6 mmol/L glucose+5 mmol/L fructose did not stimulate the rate of pHi recovery (0.2±0.9 FU/s; n=6). These data suggest that fructose stimulates the rate of pHi recovery via a PKC-dependent mechanism (Figure 2).

In the previous experimental approach, glucose was removed from the perfusate and fructose added to it. Therefore, we next performed a dose response of fructose in separate sets of experiments in which the basal rate of pHi recovery was first tested in the presence of 0.6 mmol/L glucose and in a second period, 1, 3, or 5 mmol/L fructose was added. In the presence of 1 mmol/L fructose, the rate of pHi recovery did not change significantly (0.2±0.9 FU/s; n=5) when compared with a basal pHi recovery rate in the presence of 0.6 mmol/L glucose. However, in the presence of 3 and 5 mmol/L fructose, the rate of pHi recovery increased (3.5±0.7 FU/s; n=5; P<0.02 and 2.2±0.4 FU/s; n=5; P<0.02, respectively) when compared with a basal pHi recovery rate in the presence of 0.6 mmol/L glucose (Figure 3).

To make sure that these effects were not because of merely the addition of the sugar, control experiments were performed to test the effects of a 5 mmol/L glucose-containing physiological saline on the rate of pHi recovery. In the presence of 0.6 mmol/L glucose, the basal rate of pHi recovery was 4.0±0.6 FU/s. When 5 mmol/L glucose was added to the luminal perfusate, the rate of pHi recovery did not change (3.6±0.7 FU/s; n=11; Figure S1 in the online-only Data Supplement). These results clearly indicate that fructose rather than glucose stimulates the rate of pHi recovery in the proximal tubule.

To show that luminal NHE was mediating the initial recovery of pHi after the NH4Cl acid load, we repeated the previous experiments in the absence of luminal sodium to block luminal NHE activity while keeping the sodium-containing physiological saline in the bath throughout the experiment. In the presence of a luminal sodium-free solution, basal rate of pHi...
recovery was $-0.34 \pm 0.36$ FU/s. When 5 mmol/L fructose was added to the luminal perfusate, the rate of pH$_i$ recovery was $-0.05 \pm 0.34$ FU/s ($n=5$; Figure 4). These experiments demonstrate that the initial rate of pH$_i$ recovery is because of luminal NHE activity. Furthermore, fructose does not modify the rate of pH$_i$ recovery in the absence of luminal sodium.

Finally, we tested the effect of low concentrations of angiotensin II in the presence or absence of luminal fructose. In the presence of 5.5 mmol/L glucose in the luminal perfusate, angiotensin II (1 pmol/L) did not affect the rate of pH$_i$ recovery (change, $-1.1 \pm 0.5$ FU/s; $n=9$). However, when these experiments were repeated in the presence of 0.6 mmol/L glucose+5 mmol/L fructose, angiotensin II increased the rate of pH$_i$ recovery (change, $4.0 \pm 2.2$ FU/s; $P<0.02$; $n=6$; Figure 5). These data suggest that the presence of luminal fructose enhanced the ability of angiotensin II to stimulate NHE activity in the rat proximal tubule.

Discussion

Our hypothesis was that acute fructose stimulates luminal NHE activity and sensitizes the proximal tubule to angiotensin II. Here, we directly tested the effects of acute fructose on NHE activity in isolated and perfused proximal tubules. In addition, we studied the effects of 20% fructose in the diet on blood pressure in animals fed either a normal or a high-salt diet. In this report, we provide evidence that (1) combining 20% fructose and high salt in the diet caused blood pressure to increase; (2) fructose directly stimulates NHE activity in the proximal tubule; and (3) fructose sensitizes the proximal tubule to angiotensin II.

We first studied the effects of 20% fructose in the diet on blood pressure in animals fed either a normal or a high-salt diet. At baseline, the blood pressure was similar between the experimental groups. Fructose feeding for 7 days did not change blood pressure significantly. However, adding high salt to the diet caused blood pressure to increase, and by day 7 of high-salt treatment the blood pressure of these rats reached a peak. These data show that moderate amounts of fructose in the diet can cause salt sensitivity.

Fructose has been implicated in the epidemic of diabetes mellitus, obesity, and hypertension.$^{1,2,5}$ Most of the studies addressing these effects are chronic studies in which fructose was used at 40%, 60%, or even higher concentrations for extended periods of time.$^{3,5}$ These approaches cause profound effects such as fat accumulation,$^2$ enhanced uric acid production, and insulin resistance.$^{5,7}$ Although these detrimental effects of fructose consumption have been studied for the past 20 years, the intrinsic mechanisms by which fructose induces hypertension are still unknown and acute effects of fructose in the kidney have not been thoroughly studied.

The proximal nephron reabsorbs most of the sodium, water, and bicarbonate filtered by the glomerulus. It is therefore essential in the regulation of extracellular volume homeostasis and therefore blood pressure.$^{11,12}$ Enhanced sodium reabsorption in this segment of the nephron is implicated in the
development of hypertension. In this study, we directly tested the effects of acute addition of fructose to the luminal perfusate on NHE activity in proximal tubules. We found that substituting 5 mmol/L glucose with 5 mmol/L fructose stimulated pH recovery. However, for this experimental protocol, 2 variables were modified. First, glucose was removed from the lumen. Second, fructose was added to the lumen. To show that the stimulation of pH recovery was because of fructose addition and not because of the removal of glucose, we performed a second series of experiments in which only the addition of fructose was tested. For these experiments, basal pH recovery was measured in the presence of a low glucose-containing physiological saline and then 1, 3, or 5 mmol/L fructose added to the luminal perfusate in a second period. Under these circumstances, we also found that acute addition of 3 and 5 mmol/L of fructose stimulated pH recovery. Finally, to demonstrate that the effects on pH recovery were exclusively exerted by fructose, we studied the effect of acute glucose. For these experiments, basal pH recovery was measured in the presence of 0.6 mmol/L glucose and 5 mmol/L glucose was added to the lumen in a second period. Under these circumstances, glucose did not stimulate pH recovery. These data clearly indicate that acute fructose stimulates pH recovery in the proximal tubule.

It has been shown previously that the initial recovery rate of pH after an NH4Cl acid load is mediated by luminal NHE3. Nevertheless, epithelial cells in the proximal tubules might regulate their intracellular pH by several mechanisms such as sodium/hydrogen exchange through luminal NHE3, sodium/hydrogen exchange through basolateral NHE1, and sodium/bicarbonate cotransport, which in turn depends on luminal NHE3 activity. To resolve which transporter mediated pH recovery, we performed a series of experiments where sodium ions were removed from the lumen to inhibit luminal NHE activity. Under these circumstances, the initial recovery of pH after an NH4Cl acid load in the absence or presence of luminal fructose was blocked completely. These experiments indicate that (1) the initial rate of pH recovery after an NH4Cl acid load is because of luminal NHE activity and (2) that fructose does not modify the rate of pH recovery when luminal NHE activity is inhibited. Altogether, these data suggest that fructose exerts its effects by stimulating luminal NHE activity. Because NHE3 is the predominant, if not the only NHE transporter, we report that fructose-mediated pH recovery is because of NHE3 activation.

It is noteworthy that proximal tubules were bathed with a sodium-containing physiological saline. Therefore, on the basolateral side, physiological concentrations of sodium were readily available for these cells. Finally, transcellular reabsorption of bicarbonate may play a role in recovering pH, from intracellular acidification. However, in our experiments, a HEPES-buffered physiological saline is present in the lumen of the proximal tubules. Therefore, there is no luminal bicarbonate to be titrated by extrusion of luminal protons and the mechanism of bicarbonate reclamation is absent. Given these circumstances, bicarbonate transport is unlikely to mediate the recovery of pH. These findings suggest that enhanced sodium transport in the proximal tubule might be one of the early effects exerted by fructose that would likely contribute to the development of hypertension.

In agreement with our results, Queiroz-Leite et al recently found that similar concentrations of fructose stimulate NHE3 activity in the proximal tubule of Wistar rats using in vivo stationary microperfusion. The advantages of our approach are (1) we could measure luminal NHE activity in isolated proximal tubules discarding any potential effects of the surrounding tissue and (2) our studies were done in the presence of luminal flow. This is of high physiological relevance because epithelial transport is known to be critically dependent on luminal flow in the proximal tubule. Similar to our approach, Queiroz-Leite et al also studied the recovery of pH after an NH4Cl acid load in pig kidney epithelial cells (LLC-PK1) and found that similar concentrations of fructose stimulated the rate of pH recovery. Because LLC-PK1 is a kidney proximal tubule cell line, these experiments suggested the involvement of a sodium/hydrogen exchange mechanism mediating the stimulatory effects of fructose on the rate of pH recovery. Here, we provide evidence indicating that in native proximal tubules fructose acutely stimulates the rate of pH recovery through luminal NHE activity.

Angiotensin II plays a pivotal role in blood pressure regulation because of its action on the vasculature and nephron transport. In the proximal tubule, angiotensin II displays a biphasic effect on fluid and bicarbonate reabsorption. Stimulation of transport occurs at concentrations >1 pmol/L and reaches a maximum between 10 and 100 pmol/L. At concentrations >100 pmol/L, stimulation begins to fall, such that at concentrations ≥1 mmol/L angiotensin actually inhibits transport. Therefore, 1 pmol/L was used in our studies to test whether fructose enhances the sensitivity to angiotensin II. Our results show that 1 pmol/L angiotensin II did not cause any effect on NHE activity in the presence of 5.5 mmol/L glucose. However, when this same concentration was tested in the presence of fructose in the lumen, it stimulated NHE activity. These novel findings suggest that acutely, the presence of fructose sensitizes the proximal tubule to angiotensin II. Under these circumstances, low concentrations of angiotensin II stimulate NHE activity in the proximal tubule.

Our data show that rats fed 20% fructose develop salt-sensitive hypertension when placed on a high-salt diet. Furthermore, blood pressure begins to increase immediately when dietary salt is elevated. The detrimental effects of fructose have been attributed to its chronic consumption. Chronically, a fructose-enriched diet causes obesity, insulin resistance, and hyperinsulinemia. Although fructose in the diet might not be considered acute administration of fructose, the length and concentrations of fructose used in our studies do not affect metabolic parameters commonly seen in different fructose-induced hypertension models.

Normally, a high-salt diet by itself does not raise blood pressure because animals maintain sodium balance by excreting the excess salt. Given the stimulatory effects of fructose on NHE activity in the proximal tubule, one might well ask why blood pressure was not elevated when rats were fed fructose while on a normal-salt diet. We raise the possibility here that at least part of fructose-induced salt sensitivity is a result of enhanced sodium reabsorption by the proximal tubule through both direct effects and sensitization to angiotensin II. Therefore, it is possible that fructose prevents animals to maintain sodium balance by directly enhancing sodium
reabsorption in the proximal tubule and that this effect is evidenced by increases in blood pressure when a standard sodium diet is replaced with a high-sodium diet. It is also possible that under these circumstances other compensatory mechanisms may allow the animal to maintain sodium balance preventing the increase in blood pressure.

Angiotensin II levels fall when animals are fed high salt. Consequently it is appropriate to ask whether the ability of fructose feeding to enhance the sensitivity of proximal nephron sodium reabsorption to angiotensin II might contribute to the observed salt-sensitive hypertension. We found that angiotensin II (1 pmol/L) did not affect NHE activity in the presence of luminal glucose. However, in the presence of luminal fructose, angiotensin II 1 pmol/L increased NHE activity. If fructose shifts the dose–response relationship of angiotensin II and proximal nephron sodium reabsorption, lower concentrations of angiotensin II would have greater effects. Thus, although circulating angiotensin II may fall to levels that would not enhance sodium reabsorption, in fructose-fed animals these levels could still stimulate transport.

The fact that fructose lowers the threshold for angiotensin II to stimulate NHE activity might possibly be explained by several different mechanisms. First, fructose might modify the expression of the angiotensin II type 1 receptor. However, this is unlikely because this phenomenon can be seen in 10 minutes. Second, fructose might modify the surface expression of the angiotensin II type 1 receptor. This possibility is likely to happen but unfortunately it is difficult to test because of the lack of specific antibodies against the angiotensin II type 1 receptor. Third, fructose metabolism might generate secondary metabolites, such as diacylglycerol, that further activate PKC. It has been shown that the stimulatory effects of angiotensin II on proximal tubule transport are mediated by PKC activation. Furthermore, although proximal tubules do not metabolize glucose (they are gluconeogenic), they can metabolize fructose and form diacylglycerol, one of the activators of PKC. In agreement to this possibility, our results show that the effects of fructose can be blunted by PKC inhibition. Finally, fructose might modify the surface expression of NHE3.

The rate of Na/H exchange might be modified by several parameters such as increased Na/H exchange rate of already inserted apical NHE, increased trafficking of NHE from the subapical region, increased NHE phosphorylation, or a combination of these possibilities. Nevertheless, the approach we used to measure NHE activity only provides information about the rate of sodium/hydrogen exchange. We cannot rule out any of the above-mentioned possibilities. Further studies are needed to specifically address this mechanism.

Unlike other sugars, fructose metabolism leads to intracellular ATP depletion, which in turn leads to the formation of AMP that will be further metabolized to uric acid. Hyperuricemia as a result of a long-term fructose-enriched diet participates in the development of hyperinsulinemia, hypertriglyceridemia, and endothelial dysfunction playing a critical role in the pathogenesis of fructose-induced hypertension and metabolic syndrome. Our results show that a short exposure (10 minutes) of fructose to proximal tubules stimulated the rate of pH recovery. It is therefore unlikely that a long-term consequence of fructose consumption such as increased uric acid production is involved in the acute effects of fructose. In the current work, we show that fructose exerts stimulatory effects on proximal tubular transport. Therefore, fructose might also increase uric acid transport in the proximal tubule contributing to hyperuricemia.

The concentrations of fructose in the proximal tubule are still not known. It has been reported that concentrations between 1 and 2 mmol/L can be found in the portal circulation and peripheral blood of rats and humans. Considering that the same amounts can be present in the kidney ultrafiltrate, one may assume that after water reabsorption in the first segment of the proximal nephron these concentrations might be even higher. Therefore, we would expect concentrations in the mmol/L range, which are the concentration we selected for our study. Nevertheless, the mechanisms that regulate plasma fructose levels are not completely understood yet. Recently, it has been suggested that the sodium/glucose cotransporter 5 plays a major role in reabsorbing fructose in the proximal tubule. Here, we show that PKC inhibition prevents the effects of acute fructose on pH recovery. Because PKC is activated by the generation of other intermediate metabolites, such as diacylglycerol, it is likely that fructose is first absorbed and then metabolized. Therefore, fructose reabsorption in the proximal tubule through sodium/glucose cotransporter 5 might play a role in the acute effects of fructose.

In summary, (1) combining 20% fructose and high salt in the diet caused blood pressure to increase; (2) fructose directly stimulates NHE activity in the proximal tubule likely via a PKC–dependent mechanism; and (3) fructose sensitizes the proximal tubule to angiotensin II.

Perspectives

Uncovering how fructose modifies physiological parameters within the kidney, such as proximal nephron transport and angiotensin II function, will lead us to a better understanding of the mechanisms by which salt-sensitive hypertension develops. This, in turn, may contribute to the development of new therapeutic approaches for hypertension, particularly when this disease coexists with other diseases related to fructose consumption, such as obesity and diabetes mellitus. In addition, the further knowledge of these mechanisms may serve to set new dietary parameters for the general population.

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Disclosures

None.

References

Novelty and Significance

What Is New?

Although it is clear that chronic consumption of large quantities of fructose causes several disorders, such as obesity, diabetes mellitus, and hypertension, it is less clear whether acutely, fructose modifies certain physiological parameters within the kidney that might be implicated in the development of hypertension. In addition, the intrinsic effects of fructose at the tissue level have not been studied yet. Here, we present evidence indicating that acute, fructose exerts direct effects on the proximal tubule. These effects might be implicated in the pathogenesis of fructose-induced hypertension.

What Is Relevant?

Ultimately all forms of salt-sensitive hypertension must include a renal defect or pressure natriuresis would restore normal blood pressure. In this report, we demonstrate for the first time that acute fructose stimulates luminal sodium/hydrogen exchanger activity and enhances the ability of angiotensin II to stimulate luminal sodium/hydrogen exchanger activity in the proximal tubule.

Summary

(1) Fructose (20%) feeding for 7 days did not change blood pressure significantly. Adding high salt to the diet caused blood pressure to increase; (2) fructose directly stimulates sodium/hydrogen exchanger activity in the proximal tubule likely via a protein kinase C–dependent mechanism; and (3) fructose sensitizes the proximal tubule to angiotensin II.

References


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Fructose Stimulates Na/H Exchange Activity and Sensitizes the Proximal Tubule to Angiotensin II

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Running title: Fructose stimulates NHE activity
Methods

Chemical and solutions

The pH-sensitive fluorescent dye 2′,7′-bis-(2-carboxyethyl)-5-(and-6)-
 carboxyfluorescein acetoxyethyl ester (BCECF-AM) from Molecular Probes (Eugene, OR) was
 prepared daily in DMSO and diluted in physiological saline to a final concentration of 1 µmol/L. Fructose from Sigma-Aldrich (St. Louis, MO) was prepared fresh daily. Angiotensin II (Sigma-Aldrich, St Louis, MO) was prepared in 150 mmol/L NaCl and stored as a 1 mmol/L stock solution. Before each experiment, angiotensin II was diluted in physiological saline to a final concentration of 1 pmol/L. The composition of the physiological saline was (in mmol/L): 130 NaCl, 4 KCl, 2.5 NaH2PO4, 1.2 MgSO4, 6 L-alanine, 1 trisodium citrate, 5.5 glucose, 2 calcium dilactate, and 10 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), pH 7.4 at 37°C. In some solutions either: A) glucose was reduced to 0.6 mmol/L and 4.9 mannitol was added; B) glucose was reduced to 0.6 mmol/L and 5 mmol/L fructose was added.

As previously described 1, the sodium-free solution used to perfuse the tubules contained (in mmol/l) 270 mannitol and 10 HEPES, pH 7.4 (titrated with KOH) at 37°C. The only inorganic ion present was K (≈5 mM).

The composition of the solution utilized to generate the ammonium chloride acid pulse was (in mmol/L): 120 NaCl, 10 NH4Cl, 4 KCl, 2.5 NaH2PO4, 1.2 MgSO4, 6 L-alanine, 1 trisodium citrate, 5.5 glucose, 2 calcium dilactate, and 10 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), pH 7.4 at 37°C. All solutions were adjusted to 290 ± 3 mOsm/kg H2O as measured by freezing-point depression.

Animals for in vitro studies

Male Sprague-Dawley (SD) rats weighing 100 to 150 g (Charles River Breeding Laboratories, Wilmington, MA) were fed a diet containing 0.22% sodium and 1.1% potassium (Purina, Richmond, IN) for at least 3 days prior to the experiments. All protocols and procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Case Western Reserve University in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Preparation of isolated proximal tubule segments

Rats were anesthetized with ketamine (100 mg/kg body wt i.p.) and xylazine (20 mg/kg body wt i.p.). The abdominal cavity was opened and the left kidney superfused twice with ice-cold 150 mmol/L sodium chloride, then removed and placed in physiological saline at 4°C. Coronal slices were cut and tubule segments corresponding to the proximal tubule were isolated from the kidney cortex using microforceps under a stereomicroscope at 4 to 10°C. Tubules ranging from 0.7 to 1.0 mm were transferred to a temperature-regulated chamber and perfused using concentric glass pipettes at 37 ± 1°C as we have previously done 2 3.

Measurement of intracellular pH by fluorescence microscopy
The fluorescence detection system was mounted on a Nikon Diaphot inverted microscope (Nikon, Japan). Proximal tubules were loaded by bathing the tubules with 1 µM BCECF-AM at 37 ± 1°C for 5 min and then washed for 10 min in dye-free physiological saline. The intracellular dye was excited alternately at 490 and 450 nm and emitted fluorescence measured using a 510 nm dichroic mirror. Images were recorded utilizing a 40X immersion oil objective and a Coolsnap HQ digital camera (Photometrics, Tucson, AZ), and ratiometric measurements (490/450) were recorded using Metafluor version 7 imaging software (Universal Imaging, Downington, PA).

**Measurement of NHE activity using the NH4Cl acid pulse**

After the dye-free washing period, measurements were taken once every 2 sec for 1 min. Then the basolateral bath was switched to a NH4Cl containing solution to generate intracellular acidification of proximal tubule cells. This maneuver causes an initial intracellular alkalinization. After 20 sec, the basolateral bath was exchanged back to physiological saline causing acidification of the cells. The initial rate of intracellular pH (pHi) recovery that follows this intracellular acidification was taken as a measurement of NHE activity. At this point, fructose was added to the luminal perfusate and after 10 minutes the NH4Cl acid pulse repeated. The recovery rates between both periods were compared. When the effects of angiotensin II were tested, either glucose or fructose was present in the luminal perfusate throughout the experiment. The first period was taken as a control and angiotensin II added in the second period. The initial rate of pHi recovery was quantified as fluorescent units per second (FU/sec).

**Telemetry Blood Pressure Measurement**

**Animals**

Sprague-Dawley (SD) rats (225-275g). Animal protocols were in accordance with the National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee of Medical College of Wisconsin, Milwaukee, Wisconsin, USA. Animals were fed normal rodent chow throughout the experiment and were housed under conditions of constant temperature and humidity with a 12:12h light–dark cycle. Animals were allowed to adapt to these conditions for several days before starting any experimental procedures.

In order to measure blood pressure, telemetry transmitters (Data Sciences Inc., St. Paul, MN, USA) were implanted 2 weeks prior to the experiments in rats under isoflurane anesthesia. A subcutaneous pocket between the caudal edge of the flank and the most cranial extension of the knee’s range of motion was made. The transmitter body was placed in the subcutaneous pocket with the tissue snug. The transmitter catheter inserted into femoral artery and advanced to the abdominal aorta. The incision was closed with sutures and sealed with sterile tissue adhesive to help prevent infection. The suture was removed 7 days later after the incision had completely healed. Rats were allowed to recover from surgery and were returned to individual housing. A baseline arterial pressure was recorded for 3-5 days prior to the experimental period. Blood pressure was continuously recorded throughout the experimental period.

**Effects of fructose feeding alone or in combination with high salt diet on blood pressure**
This experiment was carried out in two groups (n=5/group) of SD rats implanted with telemetry transmitters for continuous monitoring of blood pressure. After baseline data collection, rats of both groups received 20% fructose in drinking water and regular rodent chow ad libitum for 14 days and blood pressure was recorded continuously. After 7 days of 20% fructose treatment, one group of these fructose-fed rats started to receive high salt diet (8%NaCl) along with 20% fructose in drinking water.

**Statistical Analysis**

We measure the initial recovery of pH after the nadir of pH. These rates were then compared between periods. Given the variability of isolated, perfused tubule results we perform only paired experiments in which period 1 is compared to period 2. Thus the analysis did not require corrections for multiple testing. Results are presented as mean ± standard error of the mean (S.E.M). A paired Student’s t-test was used to calculate the difference between periods for the same experiment. A p value of < 0.05 was taken as significant. For telemetry blood pressure measurements: data are expressed as mean ± S.E.M. Statistical significance between measurements was determined by repeated measure one-way analysis of variance followed by Tukey’s post-hoc test using GraphPad Prism® Version 4.0 software (GraphPad Software Inc, La Jolla, CA, USA). Probability values of p <0.05 were considered significant where the critical value of P was two-sided.
References


Figure S1: Effect of adding glucose on the rate of $\Delta\text{pH}_i$ recovery in proximal tubule. Adding 5 mmol/L glucose did not modify the rate of $\Delta\text{pH}_i$ recovery (N.S=non significant; $n=5$).