The Role of Recurrent Insulin-Induced Hypoglycemia on Renal Prostanoid Production

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ABSTRACT

Aims: The present study was performed to evaluate the effect of recurrent insulin induced hypoglycemia (RIIH) on renal prostaglandin I2 (PGI2) and thromboxane A2 (TXA2) production during hypoglycemia-induced hypertension.

Study Design: In the current study, renal microdialysis was adapted as a sampling tool to evaluate the changes in renal interstitial prostanoids.

Place and Duration of Study: Department of Basic Pharmaceutical Sciences, University of Louisiana at Monroe between September 2015 – August 2016.

Methodology: Male Sprague Dawley rats were treated for two weeks with 7U/kg subcutaneous insulin injections. On the 14th day, the hypoglycemic animals were divided into three groups (7U+vehicle), (7U+captopril), and (7U+NS398), and surgeries were performed. The carotid artery, jugular vein and bladder were cannulated for hemodynamic evaluation, drugs infusion and urine collection respectively. Microdialysis probes were inserted into the renal cortex and physiological saline was infused through the probe and dialysate was harvested. A 4 hour control period was then performed and followed by a 4 hour treatment period with vehicle, captopril (12 mg/kg) or NS398.
(0.3 mg/kg) bolus infusions. The renal interstitial samples were analyzed for 6-keto-PGF1α (the stable PGII metabolite) and TXB2 (the stable TXA2 metabolite) by ELISA.

Results: During hypoglycemia, the mean arterial pressure (MAP) was increased from 92±0.54 mmHg (day 0) to 142±1.08 mmHg (day 14) as compared to the saline treated group. The hypoglycemia-induced hypertension promoted the renal production of 6-keto-PGF1α (187±31 pg/ml) and TXB2 (382±35 pg/ml) as compared to saline treated animals keto-PGF1α (69±6.0 pg/ml) and TXB2 (172±10 pg/ml). Captopril and NS398 treatment blocked 6-keto-PGF1α (90±16 pg/ml and 63±12 pg/ml respectively) when compared to the 7U+vehicle treated group (195±33 pg/ml). TXB2 was inhibited during captopril and NS398 treatment (211±28, and 128±23 pg/ml respectively) as compared to 7U+vehicle treated group (357±38 pg/ml).

Conclusion: These results demonstrated that RIHI induces prostanoids formation by renal AngII elevation which in turn enhances COX2 activity in the kidney.

Keywords: Angotensin II; COX2; hypoglycemia; microdialysis; prostanoids; RIHI.

1. INTRODUCTION

According to the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), approximately 180 thousand diabetic patients in the United States are suffering from kidney failure. Diabetes is the main cause of kidney failure, where 44 percent of all new cases were caused by diabetic complications [1]. Several studies have shown the indubitable effects of hyperglycemia on several potential mechanisms in the renal and cardiovascular systems such as the renin angiotensin aldosterone system (RAAS) and the oxidative stress pathway, which lead to renal failure and cardiovascular disorders. However, the main objective of glycemic management in diabetes mellitus is not only to achieve normal blood glucose levels, but also to prevent or reduce diabetic complications over a long period.

In type 1 and type 2 diabetes, the most serious and common adverse reaction during the management of diabetes is hypoglycemia which often occurs as patients attempt to establish euglycemia. In type1 diabetes, the intensive glycemic control can cause up to 10 hypoglycemic episodes per week with severe hypoglycemia at least once a year. Severe hypoglycemia has been determined as a cause of death in 2-4% of type1 diabetic patients [2-4]. Studies have shown hypoglycemia is also associated with the management of type2 diabetes [5]. The progressive glycemic control increases the incidence of hypoglycemia in type2 diabetic patients as well as in type1 diabetic patients. In type2 diabetes, the hypoglycemic episodes commonly occur in insulin-dependent diabetic patients as well as in patients with sulphonylurea medications, which are used as a second-line therapy in patients with type 2 diabetes [6,7]. Statistically, the prevalence of iatrogenic hypoglycemia is higher in type1 diabetic patients, and it occurs more often in insulin-dependent type 2 diabetic patients [7].

The Diabetes Control and Complications Trial (DCCT) reported that hypoglycemia increases the incidence of several micro- and macrovascular diseases including cardiovascular diseases, nephropathy, and retinopathy [8-10]. During hypoglycemia, the body activates a series of events, which elevates blood pressure and maintains normal supply glucose to the central nervous system. However, the body’s detection of hypoglycemia leads to changes in hemodynamic function, which includes increases in the systolic blood pressure, heart rate, and cardiac output [11]. A recent study demonstrated that acute and moderate hypoglycemic episodes also cause endothelial dysfunction by inducing inflammatory and thrombotic agents such as interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), plasminogen activator inhibitor-1 (PAI-1), and P-selectin [12].

RIIH was reported as a promoting factor in the activation of various deleterious mechanisms that cause hypertension and renal injury. Recently, our lab has shown that 14 days of insulin treatment or hypoglycemia could elevate AngII production producing hypertension due to Heme oxygenase I (HO-I) and carbon monoxide (CO) induction [13-15]. Moreover, AngII was reported as an essential factor in diabetic nephropathy through activation of various mechanisms that accelerate renal dysfunction [16,17]. Recently, we have found that renal ATP elevation disrupted the tubuloglomerular feedback (TGF) and led to renal AngII induction during two weeks of insulin-induced hypoglycemia [18].
In the renal microvasculature, RAAS activation induces prostaglandins (PGs) and prostacyclins (PGIs) release due to AngII type1 receptor activation, which elevates prostanoid formation, which has variant physiological and pathophysiological functions including platelet aggregation, vasodilation, vasoconstriction and urine flow [19,20]. AngII enhances prostanoids release through phospholipase A2 (PLA2) and inducible cyclooxygenase (COX2) induction at the mRNA and protein levels [21]. However, arachidonic acid release is induced by PLA2 enzymes and converted to Prostaglandin H2 (PGH2) through an interaction with cyclooxygenases. PGH2 is the precursor, which forms the active metabolites of arachidonic acid (PGE2, PGI2, PGD2, PGF2α, and TXA2) [22]. Prostanoids have various cellular and hemodynamic functions depending on receptor class and tissue location; for instance PGE2 has four different types of receptors each one has a unique signaling pathway. In the renal vasculature, PGE2 has two inverse actions as a vasodilator and vasoconstrictor based on the expression and function of its receptors (EP1, EP2, EP3, and EP4) [23,24]. On the other hand, PGF2α and TXA2 are well known as potent vasoconstrictors and regulators of renal filtration in concert with other vasodilatory prostanoids such as PGI2 and PGE2 [25].

The current study was performed to investigate the hemodynamic role and mechanism of renal cortical production of prostanoids (PGI2 and TXA2) during RIIH.

2. MATERIALS AND METHODS

2.1 Materials

Humulin was obtained from Eli Lilly and Company (Indianapolis, IN, USA). CMA 30 linear microdialysis probes were purchased from CMA/Microdialysis (Harvard Apparatus, Holliston, MA, USA). ELISA kits (6-keto-PGF1α and TXB2) were obtained from Enzo Life Sciences (Farmingdale, NY, USA). Inactin (thiobutabarbital sodium), captopril, and NS398 were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other materials were obtained from Fisher Scientific (Houston, TX, USA).

2.2 Animals

Male Sprague-Dawley rats (200-250 g, n=20, Harlan, Indianapolis, IN) were housed in controlled room temperature and light facility. Rats had free access to standard rat chow and water throughout the experiment. All animal experiments were approved by the University of Louisiana at Monroe Institutional Animal Care and Use Committee (IUCAC).

The present study was performed in accordance with a previously described 14 day RIIH animal protocol [13]. To date several studies have examined neurological and hemodynamic changes during hypoglycemia through similar methodology [13,14,26]. As previously described, male rats were subcutaneously injected with 7 units/kg of humulin insulin or saline (control) for 14 days [12]. During RIIH, blood pressure was monitored on days 0, 3, 7, 11, and 14 via tail cuff plethysmography. Blood glucose was measured on days 0, 4, 8, and 14 via a glucometer. Daily measures of water and food intake were obtained [13].

2.3 Acute Study

On the 14th treatment day, rats were anaesthetized with inactin (120 mg/kg IP) and microdialysis probes were implanted into the renal cortex, as previously described [15,27]. In the current study, we used a linear microdialysis probe to collect targeted renal interstitial samples which diffused through a semipermeable membrane (6000 Daltons cut-off) based on their concentration gradients. The inlet tube of the probe was attached to a microinfusion pump (HARVARD apparatus, PHD 2000) for saline infusion (3 µl/min). The outlet tube was connected to Eppendorf tubes for sample collections (180 µl/hour).

After surgery, a 45 min stabilization period was observed to allow hemodynamic and excretory functions to normalize. A 4 hour control period was then performed and followed by a 4 hour treatment period with vehicle, captopril (12 mg/kg) or NS398 (0.3 mg/kg) bolus infusions. Blood pressure and heart rate were recorded during entirety of the experiments. Urine flow was measured via bladder catheterization, and renal interstitial samples were collected during the control and treatment periods. All harvested samples were stored at -80°C until analyzed.

2.4 Determination of 6-keto-PGF1α and TXB2 in the Renal Interstitial Fluid

The renal interstitial samples were analyzed to determine the level of 6-keto-PGF1α (the stable PGI2 metabolite), and TXB2 (the stable TXA2
metabolite). 6-keto-PGF1α and TXB2 concentrations were determined by using commercially available colorimetric competitive enzyme immunoassay kits. The intensity of the generated yellow color was detected by a microplate reader set at 405 nm. Generated yellow color was inversely proportional to the concentration of 6-keto-PGF1α and TXB2 in either standards or the renal interstitial samples. By using Graphpad Prism Software, the unknown concentrations of 6-keto-PGF1α and TXB2 were calculated from a standard curve. The coefficient variation (CV) values for 6-keto-PGF1α and TXB2 analysis were less than 7% and 9%. Moreover, the limit of detection (LOD) values were 1.109 pg/ml and 12.70 pg/ml for 6-keto-PGF1α and TXB2 analysis, respectively.

2.5 Statistics

Data were expressed as mean ± SE and analyzed by two-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test when appropriate (INSTAT 3). P < 0.05 as considered significant.

3. RESULTS

3.1 Blood Glucose Measurement

As previously reported, blood glucose was continuously decreased during insulin treatments on Day 4, Day 8, and Day 14 (42±0.33 mg/dl, 38±0.44 mg/dl, and 31±0.25 mg/dl, respectively) as compared with saline treatment (71±0.38 mg/dl, 69±0.17 mg/dl, and 72±0.63 mg/dl respectively) (Fig. 1).

3.2 Hemodynamic Changes in Awake Rats

Mean arterial blood pressure (MAP) was measured 1 hour post insulin (7U/kg, SC) injections. A significant increase in MAP (117±0.93 mmHg) was observed after three days of insulin treatment when compared to saline treated rats (96±1.00 mmHg). MAP was increased dramatically from Day 0 to Day 14 (92±0.54 mmHg to 142±1.08 mmHg, respectively) with no significant increase in MAP in saline treated rats (97±0.66 mmHg to 98±1.64 mmHg, respectively) (Fig. 2a). Also, systolic blood pressure (SBP) (Fig. 2b), and diastolic blood pressure (DBP) were increased during RIH. There were no significant changes in heart rate (HR) during saline and insulin treatments (Fig. 3).

Fig. 1. Blood glucose measurement. Blood samples were obtained from the tail vein of both groups (saline treated, n=5, and 7U insulin treated, n=15). Blood glucose levels decreased during 7U insulin administration from day 4 to day 14. Values are expressed as mean ± SE. ***P < 0.001 for 7U insulin treated group in day 4, day 8 and day 14 when compared to saline treatment (two-way ANOVA Followed by Tukey Kramer test)

Fig. 2. Systemic hemodynamic measurement. MAP and SBP were continuously increased during RIH in day 3, day 7, day 11, and day 14 as shown in Fig. 2a and Fig. 2b respectively. Values are expressed as mean ± SE. ***P < 0.001 for 7U insulin treated group in day 3, day 7, day 11, and day 14 when compared to saline treatment (two-way ANOVA Followed by Tukey Kramer test)
3.3 Water and Food Intake

There were no significant changes in water and food intake during 14 days of insulin treatment as compared with saline treatment. Therefore, demonstrating that alterations in water and food intake did not produce the observed changes in hemodynamic or excretory functions (Table 1).

Table 1. Effect of 7U insulin administration on food and water intake

|                | Saline    | 7U Insulin |
|----------------|-----------|------------|
| Food intake    |           |            |
| (grams) Day0   | 25±0.54   | 23±0.13    |
| Day4           | 26±0.28   | 25±0.15    |
| Day8           | 26±0.71   | 24±0.23    |
| Day14          | 24±0.28   | 24±0.10    |
| Water intake   |           |            |
| (ml) Day0      | 52±1.67   | 49±0.37    |
| Day4           | 52±0.89   | 49±0.50    |
| Day8           | 46±1.09   | 47±0.35    |
| Day14          | 50±1.14   | 48±0.33    |

Values are expressed as mean ± SE. There were no significant changes observed in water and food intake in both saline and 7U insulin treated groups (two-way ANOVA Followed by Tukey Kramer test).

3.4 Hemodynamic Changes in Anaesthetized Rats

As previously described, the hypoglycemic animals (n=15) were anaesthetized and divided into three groups, where each group was treated acutely with vehicle (stock solution), captopril (12 mg/kg) or NS398 (0.3 mg/kg). We observed a significant increase in MAP in 7U+vehicle treated rats (138±0.35 mmHg) as compared with saline treated rats (122±0.07 mmHg), and 7U+vehicle treated rats (138±0.35 mmHg) (Fig. 4). There were no significant changes in MAP in 7U+NS398 treated rats (131±0.05 mmHg) as compared with 7U+vehicle treated animals (138±0.35 mmHg) (Fig. 4). There were no significant changes in heart rate during the acute studies in any of the treatment groups (Table 2).

3.5 Urine Flow Measurements

There were no significant changes in urine flow in the hypoglycemic rats during the control period (14.50±0.65 ul/min, 14.42±0.49 ul/min, and 14.23±0.75 ul/min) when compared to rats with normal blood glucose levels (12.64±0.46 ul/min) (Table 2). During the treatment period, urine flow was also not significantly changed in 7U+vehicle (14.86±0.16 ul/min), and 7U+captopril (12.29±0.11 ul/min) as compared with normal rats (13.40±0.46 ul/min) (Table 2). In the 7U+NS398 group, urine flow was reduced (8.62±0.19 ul/min) (Table 2) when compared to other treated groups. Urinary sodium (UNaV) and potassium (UKV) excretion, and glomerular filtration rate (GFR) were not changed during RIICH as shown in our previous study [13].

3.6 Prostanoids Levels in the Renal Cortex

In the present study, we observed an elevation in the renal 6-keto-PGF1α and TXB2 in all hypoglycemic hypertensive rats. In the control period, the collected renal interstitial samples
Table 2. Effect of hypoglycemia (7U insulin), 7U+captopril (12 mg/kg), and 7U+NS398 (0.3 mg/kg) on urine flow and heart rate in anesthetized rats

|                | Urine flow (ul/min) | Heart rate (BPM) |
|----------------|---------------------|------------------|
|                | 4 hrs               | 8 hrs            |
| Saline         | 12.64 ± 0.46        | 13.40 ± 0.46     | 371 ± 1.00     |
| 7U+Vehicle     | 14.50 ± 0.65        | 14.86 ± 0.16     | 361 ± 1.32     |
| 7U+Captopril   | 14.42 ± 0.49        | 12.29 ± 0.11     | 359 ± 0.84     |
| 7U+NS398       | 14.23 ± 0.75        | 8.62 ± 0.19***   | 366 ± 1.67     |

There were no significant changes in heart rate in all hypoglycemic groups with vehicle, captopril and NS398 treatment as compared to saline. Urine flow was reduced in 7U insulin + NS398 treated rats with no significant differences in 7U insulin + vehicle and 7U insulin + captopril treated rats as compared with saline treated rats. Values are expressed as mean ± SE. ***P < 0.001 for 7U+NS398 when compared with saline and 7U+vehicle treated groups during the treatment period (two-way ANOVA Followed by Tukey Kramer test).

showed a significant increase in 6-keto-PGF1α level in the hypoglycemic animal groups (187±31, 194±38, and 197±35 pg/ml) as compared to saline treated group (69±6.0 pg/ml) (Fig. 5). Captopril and NS398 treatment blocked 6-keto-PGF1α release (90±16 pg/ml and 63±12 pg/ml respectively) during hypoglycemia when compared to the vehicle (195±33 pg/ml), and saline treatment (72±6.1 pg/ml) (Fig. 5). Furthermore, renal TXB2 release was also induced dramatically in the hypoglycemic animals (382±35, 379±19, and 380±73 pg/ml) as compared with the saline treated group (172±10 pg/ml) (Fig. 6). During captopril and NS398 treatment, TXB2 production was inhibited in the hypoglycemic animals (211±28, and 128±23 pg/ml) when compared to the vehicle treated (356±38 pg/ml), and saline treated hypoglycemic animals (152±9.0 pg/ml) (Fig. 6). RIIH enhanced AngII production in the renal cortex that was significantly attenuated by captopril and selective COX2 inhibitor (NS398) treatment.

4. DISCUSSION

In the current study, RIIH promoted an increase in PGs production by inducing AngII and COX2 in the renal cortex. Captopril attenuated the elevation in the blood pressure in the hypoglycemic hypertensive rats via a decrease in circulating AngII. In the present study, we used non-diabetic rat model to avoid all autonomic and hormonal changes associated with genetic modification and streptozotocin-induced diabetes mellitus, which might cause hypertension and renal dysfunction [28-30]. However, in otherwise normal rats sustained hypoglycemia was produced by Humulin injections, which are commonly used in diabetes management and cause a similar chronic hypoglycemic condition [31,32].
sampling of the renal interstitial fluid with no disturbances in hemodynamic and cardiovascular functions [27]. During the current acute study, implantation of the microdialysis probe in the renal cortex produced no noted effects on systemic hemodynamic function due to avoidance of the traditional open abdominal surgery, which could produce an increase in blood loss [27]. As previously noted, there is a 1000 fold difference between renal AngII and circulating levels of AngII [33]. Therefore, renal microdialysis was employed in the current study to measure local acute alterations in PGI2 and TXA2 production.

![TXB2 level graph]

Fig. 6. TXB2 (the stable TXA2 metabolite) levels in renal interstitial fluid of saline, 7U+vehicle, 7U+captopril and 7U+NS398 treated groups

Values are expressed as mean ± SE. ***P < 0.001 for all 7U+vehicle and 7U+NS398 treated groups when compared to saline and 7U+vehicle treated groups respectively (two-way ANOVA Followed by Tukey Kramer test)

In the present study, we observed an elevation in PGI2 and TXA2 production during RIIH. Thus, AngII blockade and COX2 inhibition were applied to determine the mechanism of PGs induction during RIIH. Current literature supports the ability of ACE inhibitors such as captopril to block renal COX2 activity in a similar manner to COX2 selective inhibitors (nimesulide), where this effect is independent of intrarenal AngII activity [34]. With sustained elevations in intrarenal AngII activity, PGs production is induced and demonstrated a direct and indirect regulatory role on renal excretory function. Both renal PGs and RAAS synergistically alter renal blood flow and thus regulate excretory function [34-36]. In the present study, captopril treatment during hypoglycemia-induced hypertension decreased renal PGs production with no significant reduction in the urine flow. In addition RIIH had no effect on GFR as previously shown [13]. On the other hand, selective COX2 inhibition during RIIH decreased urine flow due to blocking renal PGs releases and therefore attenuated their renal hemodynamic role in the control of renal filtration [37-39]. Therefore, RAAS activation during RIIH promotes renal COX2 activity and enhances renal PGs production which acts as an essential compensatory mechanism for maintenance of normal excretory function.

5. CONCLUSION

The current study examined the role of renal prostanoids production such as PGI2 and TXA2 during RIIH. RIIH caused sustained hypertension and a significant elevation in renal PGI2 and TXA2. The hypoglycemia-induced hypotension was attenuated by captopril treatment, and the induction in renal prostanoids was modified by captopril and selective COX2 inhibitor (NS398) treatments. Therefore, alterations in renal prostanoids could be a potential mechanism for the observed decrease in renal excretory function during RIIH and a potential therapeutic target for treatment of hypoglycemia-induced hypertension.

ACKNOWLEDGEMENT

This work was supported by a NIH-LBRN grant and a Saudi Arabian Cultural Mission (SACM) fellowship.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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