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Functional interaction of AT1 and AT2 receptors in fructose-induced insulin resistance and hypertension in rats

Po-Shiuan Hsieh[a,*], Yueh-Hua Tai[a], Ching-Hui Loh[b], Kuang-Chung Shih[c], Wei-Tung Cheng[d], Chi-Hong Chu[e]

[a]*Department of Physiology and Biophysics, National Defense Medical Center, National Defense University, Taipei, Taiwan 114*
[b]*Department of Family and Community of Health, National Defense Medical Center, National Defense University, Taipei, Taiwan 114*
[c]*Department of Medicine, Division of Endocrinology and Metabolism, Taoyuan Arm General Hospital, Taoyuan, Taiwan 325*
[d]*Radiology Section, Armed Forces SongSham Hospital, Taipei, Taiwan 114*
[e]*Department of Surgery, Hualien Armed Forces General Hospital, Hualien, Taiwan 970*

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Abstract

The present study was performed to evaluate the potential role and functional interaction of angiotensin II AT1 and AT2 receptors (AT1R and AT2R) in the regulation of blood pressure and glucose homeostasis in fructose-induced insulin-resistant, hypertensive rats. Male Sprague-Dawley rats on fructose-enriched or regular diets for 4 weeks were subjected to 2-step euglycemic euinsulinemic (EEI) and euglycemic hyperinsulinemic (EHI) clamp studies with [3-^{3}H]glucose infusion. After a 40-minute basal period, selective AT1R and AT2R antagonists, losartan (LOS, 10 mg/kg IV bolus) and PD123319 (PD, 50 μg/kg/min), alone or in combination were separately given to control and fructose-fed groups in the 2 clamp periods. The results showed that during the EEI period, LOS significantly reduced the elevated blood pressure in fructose-fed rats, whereas PD further increased fructose-induced high blood pressure. Coadministration of LOS and PD did not alter the elevated blood pressure in fructose-fed rats. Administration of LOS and/or PD failed to change the blood pressure in control rats. During the EHI period, blockade of both AT1R and AT2R eliminated the insulin-induced blood pressure elevation in control and fructose-fed rats. Hepatic glucose production (HGP) did not alter among groups in the basal and EEI periods. Insulin infusion (EHI period) markedly suppressed HGP in control rats, but this suppressive effect was significantly attenuated in fructose-fed rats. LOS administration further reduced the insulin-induced suppression of HGP in fructose-fed rats. The whole-body glucose uptakes (rates of glucose disappearance, R_d) during the basal and EEI periods were similar among groups. During the EHI period, R_d was markedly increased in all groups and the magnitude of increase was significantly greater in control rats than in fructose-fed rats except those with LOS treatment. LOS treatment also redirected R_d in favor of glycolysis in fructose rats, but not in control rats, during the EEI and EHI periods. The effects of LOS on glycolysis during the 2 clamp periods and on HGP during the EHI period were reversed when PD was concomitantly administered, but PD alone did not alter glucose metabolism throughout the experiment in fructose-fed rats. Administration of LOS and/or PD did not change the glucose metabolism in control rats. Our data suggest that AT2R can counterbalance the AT1R-mediated effects on blood pressure and glucose metabolism in fructose-induced insulin-resistant, hypertensive rats. Furthermore, AT1R- and AT2R-mediated effects on blood pressure are disassociated with their actions on glucose metabolism in this hypertensive model.

1. Introduction

Insulin resistance and the accompanying hyperinsulinemia have been reported to play an important pathophysiological role in the occurrence and maintenance of essential hypertension [1,2]. Although a strong causal relationship between insulin resistance and hypertension has been demonstrated in both clinical and experimental studies [3], the possible common pathogenic denominator underlying their association remains unclear.

Insulin resistance has been demonstrated in several hypertensive animal models, including spontaneously hypertensive rats and fructose-induced hypertensive rats [4]. Fructose feeding induces hypertension accompanied by

* Corresponding author. Tel.: +886 2 87923100x18622; fax: +886 2 87924827.
E-mail address: pshsieh@hotmail.com (P.-S. Hsieh).

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hyperinsulinemia, insulin resistance, and hypertriglyceridemia in rats [5,6]. Although the precise mechanism is still undefined, the development of hypertension in fructose-fed rats has been ascribed to insulin resistance/hyperinsulinemia [5]. On the other hand, activation in the renin-angiotensin system (RAS) as reflected by an increase in the circulating angiotensin II (ANG II) levels and overexpression of tissue ANG II mRNA has been found in this hypertensive model [7,8]. Furthermore, administration of ANG II–converting enzyme inhibitors and ANG II antagonist can improve insulin sensitivity and prevent the development of hypertension in this model [9-11]. These observations suggest the implication of ANG II in the pathogenesis of hypertension and insulin resistance in fructose-fed rats. Iyer and Katovich [11] showed that either acute or chronic administration of the ANG II receptor antagonist, losartan (LOS), could ameliorate fructose-induced hypertension and insulin resistance in rats. It is recognized, however, that ANG II exerts its effects on blood pressure via 2 subtype receptors: subtype 1 (AT1R) and subtype 2 (AT2R) [12]. In vivo and in vitro studies have suggested that these 2 subtype receptors exert opposite effects on the cardiovascular system: activation of AT1R causes vasoconstriction and vascular proliferation, whereas stimulation of AT2R produces vasodilation and vascular antiproliferation [13,14]. The dual opposite actions of ANG II subtype receptors on the blood vessel complicate the use of these receptor antagonists in the pharmacological management of hypertension and related disorders and thus raise the possibility that blockade of AT1R may leave the AT2R unopposed and even stimulated. Indeed, in salt-restricted and renal-wrapped hypertensive rats with activated RAS, administration of therapeutic doses of AT1R antagonists resulted in a stimulation of the unopposed AT2R by endogenous ANG II, which in turn synergistically reduced blood pressure [15,16]. Whether AT2R activation contributes to the effects of AT1R blockade on fructose-induced hypertension and insulin resistance is unknown. Furthermore, whether AT2R is tonically active and interacts with AT1R in the regulation of blood pressure and glucose metabolism in this hypertensive model is also unclear. Thus, the present study was designed to examine the functional role of AT1R and AT2R and their possible interaction in the regulation of blood pressure and glucose homeostasis in fructose-induced hypertensive rats.

2. Materials and methods

2.1. Animals and surgical preparation

Male Sprague-Dawley rats (5-6 weeks old) were purchased from the National Laboratory Animal Breeding and Research Center, Taipei, Taiwan. The rats were housed in cages placed in an animal room with a constant temperature of 22 ± 1°C and a fixed 12-hour light-dark cycle. All animals were handled and housed according to the guidelines and manual set by the Committee of the Care and Use of Laboratory Animals of this institution.

The rats were randomly assigned to 2 groups (n = 24 each) and fed regular chow diet or fructose-enriched diet (TD89247, Teklad Primer Labs, Madison, Wis). After 4 weeks on their respective diets, rats were anesthetized with pentobarbital (50 mg/kg IP) and catheterized with micro-renathane implantation tubing (MRE 040, 0.04 in OD × 0.25 in ID; Braintree Scientific, Braintree, Mass) in the femoral artery for blood pressure measurement and blood sampling, and with MRE 033 (0.33 in OD × 0.14 in ID) in femoral vein for solution infusion. The proximal end of the tubing was sealed off and placed in a subcutaneous pocket under the scapular area of rats. After recovery from surgery for 3 to 4 days, the rats were studied only if they had restored their body weight at least to the preoperation levels. On the morning of acute study, the proximal end of the catheter was exterized, cleared, and established for intravenous and intra-arterial accesses. The following euglycemic clamp experiments were performed in rats under unanesthetized and unrestrained conditions.

2.2. Experimental designs (2-step euglycemic euinsulinemic and euglycemic hyperinsulinemic clamp experiments)

Before clamp study, rats were further divided into 4 subgroups (n = 6 each) for the following 2-step euglycemic euinsulinemic (EEI) and euglycemic hyperinsulinemic clamp (EHI) experiments: groups C and F represent control (on regular chow diet) and fructose (on fructose-enriched diet) rats treated with vehicle (saline), respectively; groupsC_L and F_L respectively represent control and fructose rats treated with a selective AT1R antagonist, LOS; groups C_{PD} and F_{PD} were control and fructose rats treated with a selective AT2R antagonist, PD123319 (PD), respectively; groups [C_{L+PD} and F_{L+PD}] separately represent control and fructose rats cotreated with LOS and PD, respectively. Experiments were started at 9:00 AM after an overnight fast (food was removed at 6:00 PM on the day before experiments). Experiments were begun with a 40-minute basal period, followed by two 90-minute clamp periods, EEI and EHI periods. The [3-^{3}H]glucose (PerkinElmer, Boston, Mass) infusion (prime 1.8 × 10^3 Bq, continuous infusion with 3.0 × 10^3, 3.0 × 10^3, and 5.9 × 10^3 Bq during basal, EEI, and EHI periods) was initiated at 70 minutes before time 0 and continued throughout the experiment. During the 2 clamp periods, LOS (10 mg/kg IV bolus at time 0) and PD (50 μg/kg per minute IV) alone or in combination were given to separate groups of rats. Saline instead of drugs was given to C and F groups during the corresponding periods. In the second clamp (EHI) period, insulin (Actrapid, Novo Nordisk, Denmark) was additionally infused to create a similar high insulin level for evaluating insulin-dependent glucose metabolism in all groups. The appropriate insulin infusion rate that creates a similar steady-state plasma insulin concentration in control and fructose-fed groups was determined in the preliminary experiment with the same

protocol. In addition, a primed, continuous intravenous infusion of 50% dextrose was begun at time 0 to quickly clamp blood glucose at the basal level. Blood samples of 0.05 mL were obtained from the femoral artery every 10 to 15 minutes to permit measurements of the plasma glucose concentrations. This allows the steady-state basal arterial glucose level to be established in 45 to 60 minutes. One hour after starting the clamp period, 2 blood samples (0.4 mL) at 30-minute intervals were collected from the femoral artery, and an average of the measurements from these 2 blood samples represented mean value in each period. The total blood volume withdrawn over the experimental period was controlled at an amount of no more than 3 mL/rat and an equal volume of saline was simultaneously administered via the infusion line. Thus, the total blood loss was much less than the amount (7 mL/kg) reported previously to provoke stress and insulin resistance in glucose clamp study in anesthetized rats [17].

2.3. Blood pressure measurements

Mean arterial blood pressure (MAP) and heart rate were directly measured in rats under unanesthetized state during experiment by connecting the femoral artery catheter to a polygraph (Gould RS3400 4-channel recorder, Gould Inc, IGM Valley View, Ohio) via a pressure transducer (Spectramed P23XL, Spectramed Inc, Oxnard, Calif) for arterial pressure and heart rate monitoring.

2.4. Chemical analyses

Plasma glucose levels were assayed by the glucose oxidase method with a YSI glucose analyzer (YSI 2300 Plus, Yellow Springs Instruments, Yellow Springs, Ohio). Plasma triglyceride concentrations were determined by using an enzymatic colorimetric method. Plasma insulin levels were measured by the solid-phase 2-site enzyme immunoassay technique using a commercially available rat insulin enzyme-linked immunosorbent assay kit (ALPCO, Uppsala, Sweden).

2.5. Calculations

2.5.1. The whole-body glucose uptake and hepatic glucose production

A steady-state plateau of plasma [3-^{3}H]glucose–specific activity was achieved during the last 30 minutes of [3-^{3}H]glucose infusion in the basal, EEI, and EHI periods in each experiment. During the steady-state period, the rate of glucose appearance (R_a) equals the rate of glucose disappearance (R_d), and the glucose turnover rate was assessed by tracer dilution method [18]. R_d equals the rate of whole-body (WB) glucose uptake. During the basal and EEI periods, R_d equals R_a, which equals the rate of hepatic glucose production (HGP). In the insulin-stimulated state (EHI period), R_d equals the rate of HGP plus the rate of exogenous glucose infusion. Therefore, HGP equals R_d minus exogenous glucose infusion.

2.5.2. Whole-body glycolysis and whole-body glucose storage

^{3}H in the C-3 position of glucose is lost selectively to H_2O during glycolysis. Therefore, the plasma tritiated counts are present either as ^3H_2O or as [3-^{3}H]glucose [19]. Rates of WB glycolysis were determined from the increment per unit time in ^3H_2O (dpm l^{-1} min^{-1}) multiplied by the total body water mass and divided by the [3-^{3}H]glucose-specific activity (dpm/mg) [20]. Plasma water is assumed to be 93% of the total plasma volume, and total body water mass is assumed to be 65% of the body weight in control and fructose-treated groups [20,21]. The rate of WB glycolysis was determined during the last 30 minutes of [3-^{3}H]glucose infusion in each clamp period. The appearance of ^3H_2O in plasma over that period was linear in all experiments as judged by linear regression analysis. This was in agreement with previous study [21]. The rate of WB glucose storage was calculated by subtracting the rate of WB glycolysis from the rate of WB glucose uptake (R_d). The calculated rate of WB glucose storage primarily reflects the glycogen synthesis [20] plus a relatively ignorable amount of glucose that is converted to lipid [22].

2.6. Statistical analysis

Statistical analysis was performed according to the repeated measurements of 1-way analysis of variance (ANOVA) followed by Bonferroni test. A probability of $P < .05$ was taken to indicate a significant difference between means. Values are expressed as means ± SEM.

3. Results

3.1. Body weight, plasma hormone, glucose concentrations, and [3-^{3}H]glucose-specific activity

There were no significant differences in the body weight and plasma glucose levels among groups throughout the experiments (Table 1). During the basal and EEI periods, plasma insulin levels were about 3-fold higher in fructose-fed rats than in control rats. During the EHI period, additional insulin infusion created a comparable high insulin level (about 10-fold basal insulin level of C) in all groups. The [3-^{3}H]glucose-specific activity remained similar among groups throughout the experiments. Insulin and glucose infusion rates were significantly lower in fructose-fed groups than in the control group during EHI period, but no significant differences were found among fructose-fed groups (data not shown).

3.2. Mean arterial blood pressure and heart rate

Rats fed fructose diet for 4 weeks significantly increased the MAP in all fructose-fed groups (Fig. 1). During the EEI period, LOS administration markedly reduced the MAP of fructose-fed rats from 125 ± 1 to 108 ± 3 mm Hg in F_L ($P <$ .05). In contrast, PD infusion further increased fructose-

Table 1
Body weight, plasma insulin and glucose levels, and [3-^{3}H]glucose-specific activity during basal and 2 euglycemic clamp periods

		C	C_L	C_{PD}	C_{L+PD}	F	F_L	F_{PD}	F_{L+PD}
BW (g)		465 ± 23	455 ± 17	463 ± 16	463 ± 16	467 ± 12	468 ± 16	450 ± 15	461 ± 19
INS (pmol/L)	BA	233 ± 17	202 ± 24	226 ± 16	237 ± 13	637 ± 63*	622 ± 59*	723 ± 27*	674 ± 36*
	EEI	207 ± 9	214 ± 23	216 ± 13	236 ± 10	687 ± 60	603 ± 28	661 ± 43	744 ± 34
	EHI	2408 ± 118	2422 ± 72	2394 ± 80	2465 ± 98	2331 ± 77	2269 ± 93	2277 ± 54	2306 ± 137
PG (mmol/L)	BA	7.3 ± 0.3	7.1 ± 0.2	6.8 ± 0.1	7.1 ± 0.1	7.9 ± 0.2	7.9 ± 0.2	7.5 ± 0.1	7.7 ± 0.1
	EEI	6.6 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	7.0 ± 0.1	7.3 ± 0.2	7.0 ± 0.1	7.3 ± 0.2	7.0 ± 0.1
	EHI	7.2 ± 0.3	7.2 ± 0.1	6.9 ± 0.1	7.3 ± 0.1	7.1 ± 0.2	7.5 ± 0.2	7.0 ± 0.2	7.3 ± 0.2
SA ($\times 10^3$ dpm/mg)	BA	3.2 ± 0.1	3.0 ± 0.1	3.3 ± 0.1	3.0 ± 0.1	3.0 ± 0.1	3.2 ± 0.2	3.3 ± 0.1	3.2 ± 0.1
	EEI	3.3 ± 0.1	3.0 ± 0.1	2.7 ± 0.1	3.2 ± 0.1	3.0 ± 0.1	3.5 ± 0.2	3.4 ± 0.1	3.1 ± 0.1
	EHI	2.8 ± 0.2	2.9 ± 0.1	2.5 ± 0.1	2.9 ± 0.1	3.3 ± 0.1	3.1 ± 0.2	3.2 ± 0.1	3.2 ± 0.1

BW indicates body weight; INS, insulin; PG, plasma glucose; BA, basal period; SA, specific activity. Data are means ± SEM.
* $P < .05$ vs C in the basal period.

induced elevation in MAP in F_{PD} (from 126 ± 1 to 137 ± 5 mm Hg, $P < .05$). However, in F_{L+PD}, the depressor effect of LOS and the pressor effect of PD were not exhibited when both agents were given in combination. In control rats, LOS and/or PD administration did not significantly alter the MAP levels in the EEI period. During the EHI period, insulin infusion significantly increased blood pressure in all groups except those treated with combined LOS and PD. Coadministration of LOS and PD in control and fructose-fed rats prevented the insulin-mediated pressor effect during the EHI period. The heart rate was not significantly changed throughout the experiments in control and fructose-fed rats except the F_L group during the EEI period.

3.3. Hepatic glucose production, whole-body glucose uptake (Rd), glucose storage, and glycolysis

Hepatic glucose productions were not significantly different among groups during the basal and EEI periods (Figs. 2 and 3). During the EHI period, increased plasma insulin markedly suppressed HGP in control groups, but the

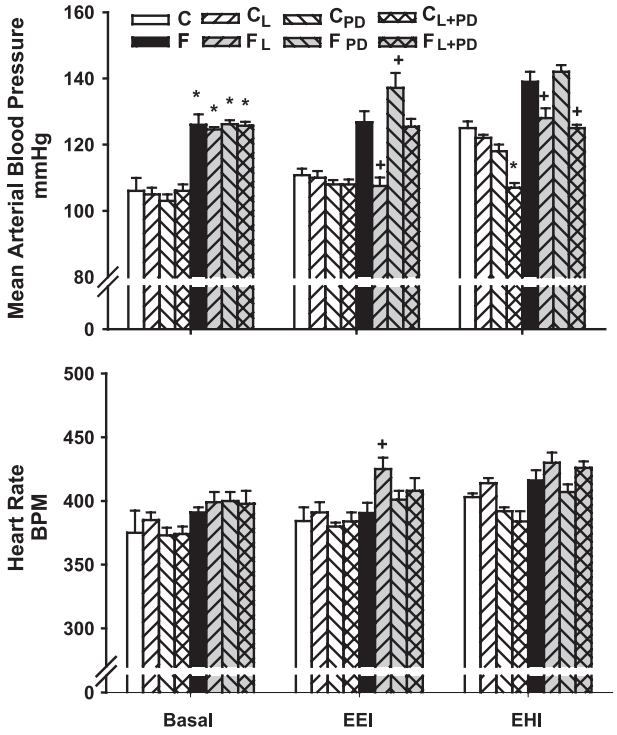

Fig. 1. The MAP and heart rate in the basal, EEI, and EHI periods of control (on regular diet) and fructose-fed rats with or without drug treatment. C indicates control rats (on regular chow diet) with vehicle; C_L, control rats with LOS; C_{PD}, control rats with PD123319; C_{L+PD}, control rats with LOS and PD123319; F, fructose-fed rats with vehicle; F_L, fructose-fed rats with LOS; F_{PD}, fructose-fed rats with PD123319; F_{L+PD}, fructose-fed rats with LOS and PD123319; BPM, beat per minutes. N = 6 per group. Asterisk indicates $P < .05$ vs C in the corresponding time point; cross, $P < .05$ vs F in the corresponding time point.

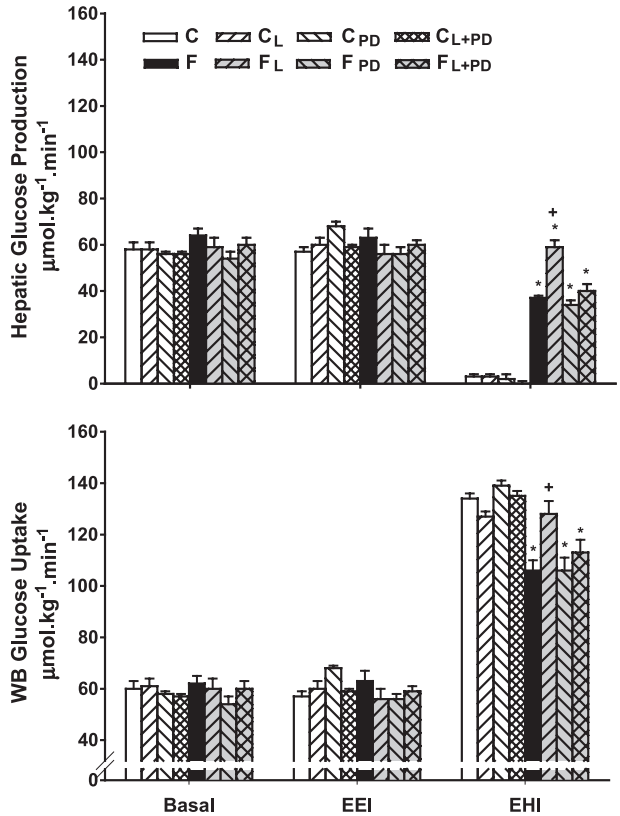

Fig. 2. The HGP and WB glucose uptake in the basal, EEI, and EHI periods of control rats on regular diet and fructose-fed rats with or without drug treatment. For abbreviations, see Fig. 1. N = 6 per group. Asterisk indicates $P < .05$ vs C in the corresponding time point; cross, $P < .05$ vs F in the corresponding time point.

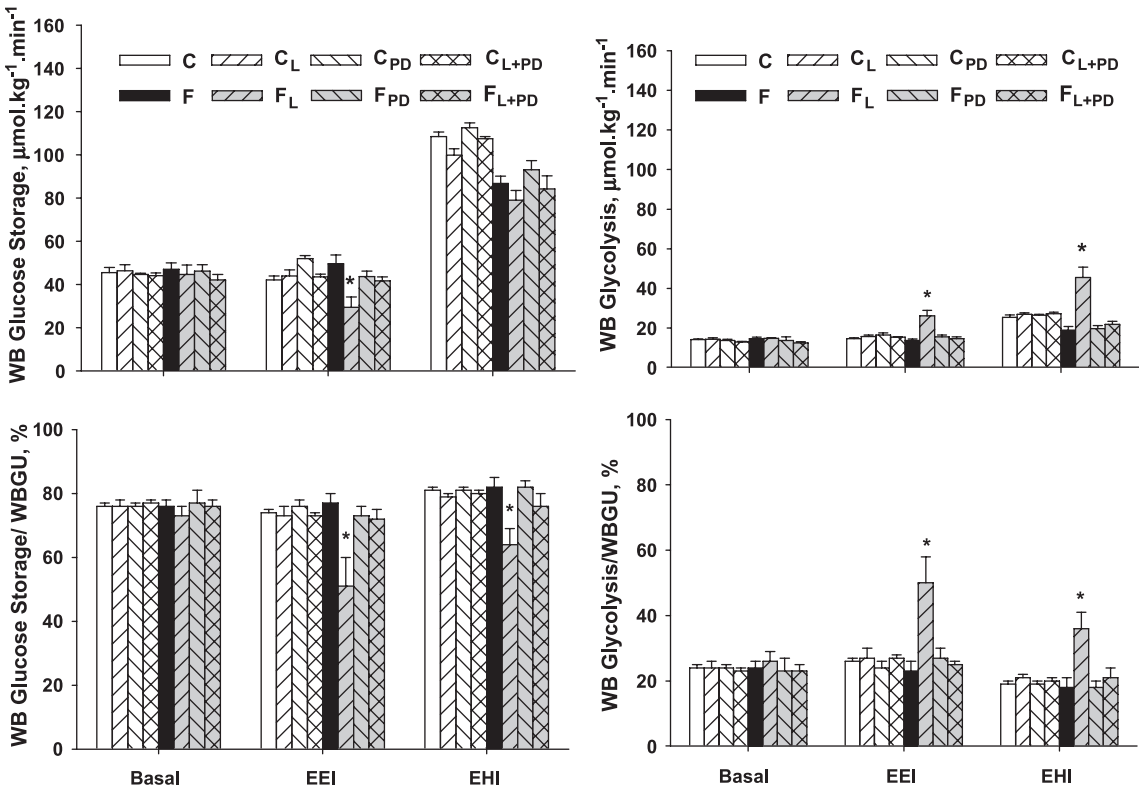

Fig. 3. Changes in WB glucose storage and WB glycolysis in the basal, EEI, and EHI periods of control rats on regular diet and fructose-fed rats with or without drug treatment. WBGU indicates the rate of WB glucose uptake. For other abbreviations, see Fig. 1. N = 6 per group. Asterisk indicates $P < .05$ vs C in the corresponding time point; cross, $P < .05$ vs F in the corresponding time point.

insulin-induced HGP suppression was significantly attenuated in fructose-fed groups except that with LOS treatment. Administration of LOS further reduced the insulin-mediated suppressive effect on HGP in fructose-fed rats and this diminished suppression of insulin on HGP by LOS was reversed when rats were concomitantly treated with PD and LOS. PD treatment alone, however, did not significantly alter HGP in fructose-fed rats. Administration of LOS and/or PD did not change HGP in control rats throughout the experiment (Fig. 2, upper panel).

During the basal and EEI periods, R_d remained similar in all groups. During the EHI period, exogenous insulin infusion significantly increased R_d in all clamp experiments and the augmented R_d was significantly less in F than in C (106 ± 4 vs 134 ± 2 μmol kg^{-1} min^{-1} for F vs C, $P < .05$). Losartan but not PD treatment further raised R_d in fructose-fed rats (125 ± 4 μmol kg^{-1} min^{-1} for F_L, $P < .05$ vs F) and this LOS-induced increment in R_d was not observed when PD was superimposed on LOS (113 ± 5 μmol kg^{-1} min^{-1} for F_{L+PD}). Losartan and/or PD treatment did not change the WB glucose uptake during the EEI and EHI periods in control rats (Fig. 2, lower panel).

The WB glucose storage (nonglycolytic glucose disposal) and glycolysis were not different between the basal and EEI periods in all groups except fructose-fed rats with LOS treatment. From the basal period to the EEI period, LOS administration alone significantly reduced the WB glucose storage flux from 44.6 to 29.4 ± 4.8 μmol kg^{-1} min^{-1} ($P < .05$) and simultaneously increased the WB glycolysis flux from 14.8 ± 0.4 to 26.2 ± 2.7 μmol kg^{-1} min^{-1} ($P < .05$) in fructose-fed rats. During the EHI period, insulin infusion significantly increased the WB glucose storage and glycolysis in all groups. The increments in WB glucose storage and glycolysis were significantly lower in fructose-fed groups than in control groups except fructose-fed rats with LOS treatment. The increment in WB glycolysis was significantly higher in fructose-fed groups with LOS treatment than in other groups. The ratio of WB glucose storage to R_d and that of WB glycolysis to R_d exhibited a similar trend, suggesting that LOS significantly decreased the WB glucose storage flux and increased the WB glycolysis flux simultaneously in fructose-fed rats, but not in control rats, under basal and hyperinsulinemic conditions. Superimposed PD on LOS reversed the LOS-mediated actions on WB glucose storage and glycolysis in fructose-fed rats (Fig. 3).

4. Discussion

Activation in the RAS has been postulated to contribute to the development of fructose-induced insulin resistance and hypertension in rats [6,8-10]. However, ANG II exerts its cardiovascular actions via at least 2 receptors, AT1R and AT2R, and these 2 subtype receptors mediate functionally

opposite effects on the vascular tone and proliferation. The linkage of the functional activation and interaction of AT1R and AT2R to the cardiovascular and metabolic dysfunctions in fructose-fed rats are not clear. The present results demonstrated that acute AT1R antagonism by LOS administration significantly reduced blood pressure and redirected the WB glucose uptake flux in favor of glycolysis under basal and hyperinsulinemic conditions in fructose-fed rats. Conversely, AT2R-selective antagonism by PD induced a further increase in blood pressure, but did not affect the basal and insulin-dependent glucose metabolism in rats on fructose-enriched diet. The effects of LOS on blood pressure and glucose metabolism were blunted by combined treatment with LOS and PD. Analogously, the pressor effect of PD was eliminated by concomitant treatment of LOS and PD in fructose-fed rats. These observations suggest that activation and functional interaction between AT1R and AT2R are implicated in the tonic control of blood pressure and glucose metabolism in fructose-induced insulin-resistant and hypertensive rats.

In agreement with previous studies [5,6,8-11], the present study demonstrated that fructose feeding for 4 weeks significantly increased blood pressure in rats and that fructose-induced hypertension could be reversed to normal blood pressure level by AT1R antagonism. In contrast, blockade of AT2R by PD administration further increased the elevated blood pressure in fructose-fed rats. Interestingly, the depressor effect of LOS could be offset by the pressor action of PD since combined treatment with LOS and PD failed to alter the elevated blood pressure of fructose-fed rats. However, the vasoactive effects of LOS and PD seen in fructose-fed rats were not exhibited in rats on regular chow diet in our study. These observations suggest that activated AT1R and AT2R only occurred in rats with fructose-induced hypertension and insulin resistance but not in rats on normal diet. In agreement with the present results, Siragy and Carey [16] demonstrated in rats with angiotensin II–dependent renal wrap hypertension that blockade of AT1R could normalize blood pressure and this effect was prevented by AT2R blockade. In sodium-restricted rats [15], AT1R blockade with valsartan reduced blood pressure together with a marked increase in the renal production of bradykinin, nitric oxide, and cyclic guanosine monophosphate. AT2R blockade with PD offset both the hypotensive and renal autacoid responses to valsartan. Taken together, these observations suggest that when RAS is activated in pathophysiological conditions, tonic stimulation of AT2R counteracts, at least partly, the activated AT1R-mediated increase in blood pressure. Blockade of AT1R by LOS treatment eliminates the elevated blood pressure, whereas inhibition of AT2R by PD exaggerates the hypertensive conditions. Thus, the depressor effect of AT1R antagonism seems mediated partly via the action of unmasked AT2R in these hypertensive animal models. How the reciprocal antagonism of blood pressure regulation between LOS and PD occurred and what mechanism maintained the elevated blood pressure during combined AT1 and AT2 blockade in fructose-fed rats remain undefined. Whether other vasoactive factors predominantly took over the control of blood pressure under this condition remains to be clarified.

There is evidence that ANG II increases insulin sensitivity in diabetic and healthy subjects, even at subpressor doses [23,24]. However, the contribution of AT1R- and AT2R-mediated metabolic actions to glucose homeostasis under insulin-resistant state has not been evaluated. To the best of our knowledge, our study demonstrated for the first time that acute LOS treatment not only increased insulin-dependent glucose uptake but also redirected glucose uptake flux in favor of glycolysis at the expense of glucose storage under basal and hyperinsulinemic conditions in rats with fructose-induced insulin resistance. We found that AT2R blockade eliminated the metabolic effect of acute LOS administration. These observations suggest that acute LOS treatment might enhance the activation of glycolytic key enzymes to redirect the WB glucose uptake flux. It is likely that this acute LOS effect was mediated via unmasking an AT2R-mediated action on the bradykinin/nitric oxide system in this hypertensive model as previously suggested [16]. A subsequent increase in kinin activity might in turn contribute to the acute effect of LOS on glycolysis. Bradykinin has been reported to significantly increase glycolytic flux via increasing fructose 2,6-biphosphate content and 6-phosphofructo-2-kinase activity in human fibroblasts [25]. Nevertheless, the possible involvement of bradykinin in LOS-mediated increase in glycolysis needs to be further elucidated.

Using the same insulin-resistant model, Iyer and Katovich [11] showed that acute LOS treatment was effective in lowering both blood glucose and plasma insulin profiles during an intravenous glucose tolerance test. They suggested that acute blockade of AT1R ameliorated glucose intolerance and insulin resistance in fructose-fed rats. Henriken et al [26] conducted study in obese Zucker rats (an animal model of obesity-associated insulin resistance) and demonstrated that the selective AT1R antagonism (irbesartan), either acutely or chronically, improved glucose tolerance, partly because of enhanced skeletal muscle glucose transport. These observations implicate that inhibition of AT1R activation can affect the glucose homeostasis under insulin-resistant state. Our present results further suggest that after acute blockade of AT1R, stimulation of the unmasked AT2R by excess ANG II might play an important role in modulating the glucose metabolism under insulin-resistant state.

The present study revealed that LOS treatment alone further attenuated the suppression effect of insulin on HGP in fructose-fed rats. This phenomenon was not seen in fructose-fed rats with combined PD and LOS treatment. Previous study has shown that ANG II has an inhibitory effect on insulin-mediated glucose metabolism by suppress-

ing phosphoinositide-3′ -kinase activity, which is blocked by saralasin (a nonselective ANG II antagonist) but not LOS [27]. It is possible that LOS treatment might have unmasked an AT2R-mediated inhibitory effect of ANG II on insulin-induced suppression of glucose production in liver. Alternatively, since blockade of AT1R results in sympathetic activation in rats [28], the increased catecholamines could also counterbalance the LOS-mediated action on HGP.

Our data also demonstrated that the actions of LOS and PD on blood pressure were not associated with their effects on basal and insulin-dependent glucose metabolism. Several clinical studies have shown that a pressor or even a subpressor dose of ANG II can produce metabolic effects [23,24]. For instance, Buchanan et al [23] found that ANG II increased insulin-stimulated glucose disposal accompanying redistribution of blood flow away from insulin-independent tissue like kidneys and a net increase in perfusion of insulin-sensitive tissues such as skeletal muscles of normal volunteers. Morris et al [24] showed that both subpressor dose (1 ng/kg per minute) and pressor dose (5 ng/kg per minute) of ANG II infusion significantly increased insulin sensitivity, with or without an increase in blood pressure, in normotensive patients with type II diabetes. These investigations suggest that the metabolic and pressor effects of ANG II might not be solely due to hemodynamic alterations and redistribution of blood flow in insulin-sensitive tissues. The present results support the contention that ANG II via AT1R and AT2R exerted separate effects on blood pressure and on glucose metabolism in fructose-induced insulin-resistant, hypertensive rats. It seems that the blood pressure responses to AT1R and AT2R blockades might be different from the metabolic responses to these ANG II receptor antagonists in insulin-sensitive vs insulin-resistant states.

Collectively, our data suggest that tonical activations of both AT1R and AT2R play a counterregulatory role in the control of blood pressure and glucose homeostasis in fructose-induced insulin-resistant, hypertensive rats. Our observations also suggest that stimulation of the unopposed AT2R is permissive in the antihypertensive and metabolic actions of acute AT1R antagonist treatment in this hypertensive model. Although the role of AT2R in long-term AT1R blockade remains to be determined, our present results implicate that AT2R should be considered as a novel potential target for the management of hypertension associated with insulin-resistant state.

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