The effect of high-fructose intake on the vasopressor response to angiotensin II and adrenergic agonists in Sprague-Dawley rats

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Abstract: Effect of losartan was assessed on systemic haemodynamic responses to angiotensin II (Ang II) and adrenergic agonists in the model of high-fructose-fed rat. Twenty-four Sprague-Dawley (SD) rats were fed for 8 weeks either 20% fructose solution (FFR) or tap water (C) *ad libitum*. FFR or C group received losartan (10mg/kg/day p.o.) for 1 week at the end of feeding period (FFR-L and L) respectively, then the vasopressor responses to Ang II, noradrenaline (NA), phenylephrine (PE) and methoxamine (ME) were determined. The responses (%) to NA, PE, ME and Ang II in FFR were lower (p<0.05) than C (*FFR vs. C*; 22±2 vs. 32±2, 30±3 vs. 40±3, 9±1 vs. 13±1, 10±1 vs. 17±1) respectively. L group had blunted (p<0.05) responses to NA, PE, ME and Ang II compared to C (*L vs. C*; 26±2 vs. 32±2, 30±3 vs. 40±3, 7±0.7 vs. 13±1, 5±0.4 vs. 17±1) respectively. FFR-L group had aggravated (p<0.05) response to NA and ME, but blunted response to Ang II compared to FFR (*FFR-L vs. FFR*; 39±3 vs. 22±2, 11±1 vs. 9±1, 3±0.4 vs. 10±1) respectively. Fructose intake for 8 weeks results in smaller vasopressor response to adrenergic agonists and Ang II. Data also demonstrated an important role played by Ang II in the control of systemic haemodynamics in FFR and point to its interaction with adrenergic neurotransmission.

Keywords: Fructose; Sprague-Dawley rats; noradrenaline; haemodynamics; angiotensin II.

INTRODUCTION

High fructose consumption is reported to produce hypertension, hyperinsulinemia, hypertriglyceridemia (Hwang *et al*., 1987). Furthermore, insulin-induced stimulation of the sympathetic nervous system is suggested as one of the mechanisms that link insulin resistance and hypertension. The chronic activation of the sympathetic nervous system brought by hyperinsulinaemia in this model may contribute to insulin resistance through enhanced vasoconstriction (Tran *et al*., 2009). Higher circulating level of catecholamines in metabolic syndrome rat on the other hand through their vasoconstrictor and/or antinatriuretic properties results in hypertension (Johnson *et al*., 1993). In addition, Ang II is also suggested to play a key role in the elevation of blood pressure and resistance to insulin action induced by fructose feeding through a mechanism that is not dependent on vascular hyperreactivity or endothelial dysfunction (Navarro-Cid *et al*., 1995).

Evidence is available on the synergistic interaction between the vascular AT_1-receptors and α_1-adrenoceptors (Abdulla *et al*., 2009b, Abdulla *et al*., 2011). A number of *in vivo* studies suggested that Ang II enhances the activity of adrenergic receptors which results in increased vasoconstriction (De Jonge *et al*., 1983, Marano and Argiolas, 1994). Moreover, Ang II is known to induce transcription and expression of α_1-adrenergic receptors in rat vascular smooth muscle cells (Hu *et al*., 1995). The blockade of endogenous Ang II by AT_1 blockers alters vascular reactivity to exogenous noradrenaline (Raasch *et al*., 2004, Abdulla *et al*., 2009b). In addition, the interaction between Ang II and adrenergic neurotransmission was well proven to play an important role in modulation of the vascular reactivity in some pathological states in rat (Rathore *et al*., 2009, Abdulla *et al*., 2009a).

It is not known whether the interaction between vascular AT_1-receptors and α_1-adrenergic receptors modulates vasomotor function in fructose-fed rats. Accordingly, the present study was undertaken to compare the vasopressor responses to intravenous administration of Ang II and adrenergic agonists in fructose-fed rats receiving chronic treatment with losartan. We hypothesized that fructose intake for 8 weeks results in blunted systemic vascular responses to adrenergic agonists and Ang II. Furthermore, normal vasomotor activity depends on intact renin-angiotensin system (RAS) and that the AT_1-receptor–blocking effects of losartan would be associated with decreased vasopressor responses to adrenergic agonists.

MATERIALS AND METHODS

Animals

Twenty-four male Sprague-Dawley (SD) rats were obtained at (155-175 g) body weight from the Central Animal Facility at Universiti Sains Malaysia, Penang,

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Malaysia, and permitted a 7-d period-of-adaptation to the environment, with free access to water and standard rodent chow (Gold Coin Sdn. Bhd., Penang, Malaysia) and tap water *ad libitum*. Thereafter, the rats were randomly assigned to control group (C; n=6) and received standard rodent chow; fructose-fed rats (FFR; n=6) were fed a standard rodent chow, and fructose was administered as a 20% solution (prepared freshly every day) in drinking water; losartan treated group (L; n=6), received losartan at 10 mg/kg/day by oral gavage for 7 days after 7 weeks period; fructose-fed rats treated with losartan (FFR-L; n=6) were fed a standard rodent chow, and received 20% fructose in drinking water in addition to 10 mg/kg/day losartan by oral gavage for 7 days at the end of fructose feeding period. All groups were followed for 8 wk. Experiments were approved by the Ethics Committee of Universiti Sains Malaysia.

Animal surgical preparation

Vasopressor responses

The overnight fasted rats were anaesthetized with 60 mg/kg i.p. sodium pentobarbitone (Nembutal®, CEVA, Libourne, France). Immediately after induction of anaesthesia the trachea was exposed through a mid-line incision and cannulated. After tracheostomy, the carotid artery was cannulated (PE 50, Portex, Kent, UK) and connected to a pressure transducer (P23 ID Gould, Statham Instruments, UK) coupled to a computerized data acquisition system (PowerLab®, ADInstruments, Sydney, Australia) for the continuous monitoring of blood pressure throughout the experiment. The left jugular vein was cannulated (PE 50, Portex, Kent, UK) and the cannula was attached to a 50 ml syringe on an infusion pump (Perfusor secura FT 50 ml, B. Braun) that delivers normal saline at a rate of 3 ml/hr throughout the experiment. The vasoactive agonists or maintenance doses of anaesthetic were injected through the infusion line. The vasopressor response experiment was performed as follows: After a stabilization phase of 1 hour, baseline systemic haemodynamic values were obtained. Those include systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MAP), pulse pressure (PP) and heart rate (HR). This was followed by the recording of acute vasopressor responses to the IV administration of noradrenaline (NA; 200, 400 and 800 ng), phenylephrine (PE; 2, 4, and 8 µg), methoxamine (ME; 2, 4 and 8 µg) and angiotensin II (Ang II; 5, 10 and 20 ng). All vasoactive agonists were administered twice at the above doses, firstly, from lowest to highest dose and secondly, from highest to lowest dose, and the average value of response for each dose was taken. Sequential bolus injections were to produce pressure responses ranging from 5 to 50 mm Hg. Consecutive administrations of the agonists were separated by a period of 10 min to allow a full recovery of mean arterial blood pressure and heart rate. Doses of all vasoactive stimuli were from previous studies in this lab (Rathore *et al*., 2009, Abdulla *et al*., 2010).

Vasoactive agents

Noradrenaline (Sanofi Winthrop, Surrey, UK), Phenylephrine (Knoll, Nottingham, UK), methoxamine (Wellcome, London, UK) and angiotensin II (CIBA-GEIGY, Basel, Switzerland) were used in the haemodynamic study. All of these drugs were prepared as stock solutions in normal saline and stored at +4°C. Noradrenaline vasoconstrictor response is suggested to be mediated by α_1-adrenoceptors (Zacharia *et al*., 2004). Further, phenylephrine non-selectively binds to α_1-adrenoceptors (Armenia *et al*., 2004) and produces vasoconstriction, while methoxamine vascular response is due to its selective binding to α_{1A}-adrenoceptors (Tsujimoto *et al*., 1989). Ang II produces vasoconstriction due to binding to AT1 receptors.

STATISTICAL ANALYSIS

The vasopressor responses caused by Ang II and adrenergic agonists, were taken as the average values caused by each dose of each agonist administered in ascending and descending orders. The overall mean response for each dose was taken as the average value of the vasopressor responses (% change in MAP) obtained at that dose. All data presented in this study are expressed as mean change±SEM of vasopressor responses elicited by all doses of each agonist and have compared between the treated groups and a respective control. The statistical analysis of data was done by one-way ANOVA followed by Bonferroni *post hoc* test using the statistical package Superanova (Abacus In., CA, USA). The differences between the means were considered significant at 5% level.

RESULTS

Baseline systemic haemodynamic values

The baseline values of SBP, MAP and HR in FFR were higher (all $p<0.05$) than C, while baseline DBP in FFR was similar to C (table 1). In addition, losartan treatment had no significant effect on baseline haemodynamic values in C except that HR in L was higher than C. FFR-L group had significantly ($p<0.05$) lower values of SBP and MAP compared to FFR (table 1).

Vasopressor responses

Exogenously administered NA, PE, ME and Ang II resulted in dose-dependent increases in MAP in all groups (fig.1). The vasopressor responses to NA, PE, ME and Ang II (expressed as (%) increase in MAP) in FFR were significantly lower (all $p<0.05$) than C (*FFR*: NA, 22±2; PE, 30±3; ME, 9±1; Ang II, 10±1 vs. *C*: NA, 32±2; PE, 40±3; ME, 13±1; Ang II, 17±1) (fig.2). In addition, losartan treatment produced lower vasopressor responses to Ang II and adrenergic agonists compared to C (*L*: NA,

Table 1: Baseline systemic haemodynamic values

Group	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)	PP (mmHg)	HR (bpm)
C	116±3	88±3	112±3	28±2	245±3
FFR	148±5*	118±5	133±5*	30±1	364±17*
L	127±7	100±7	113±7	28±1	353±15*
FFR-L	113±12#	86±14	99±13#	27±3	310±37

Baseline systemic haemodynamic values measured during the acute vasopressor response experiment in control (C), fructose-fed (FFR), losartan treated (L) and fructose-fed losartan treated (FFR-L) rats. Values are mean±SEM of n = 6 rats in each group. * Indicates $p<0.05$ vs. C, # indicates $p<0.05$ vs. FFR. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; PP, pulse pressure; HR, heart rate; bpm, beat per minute.

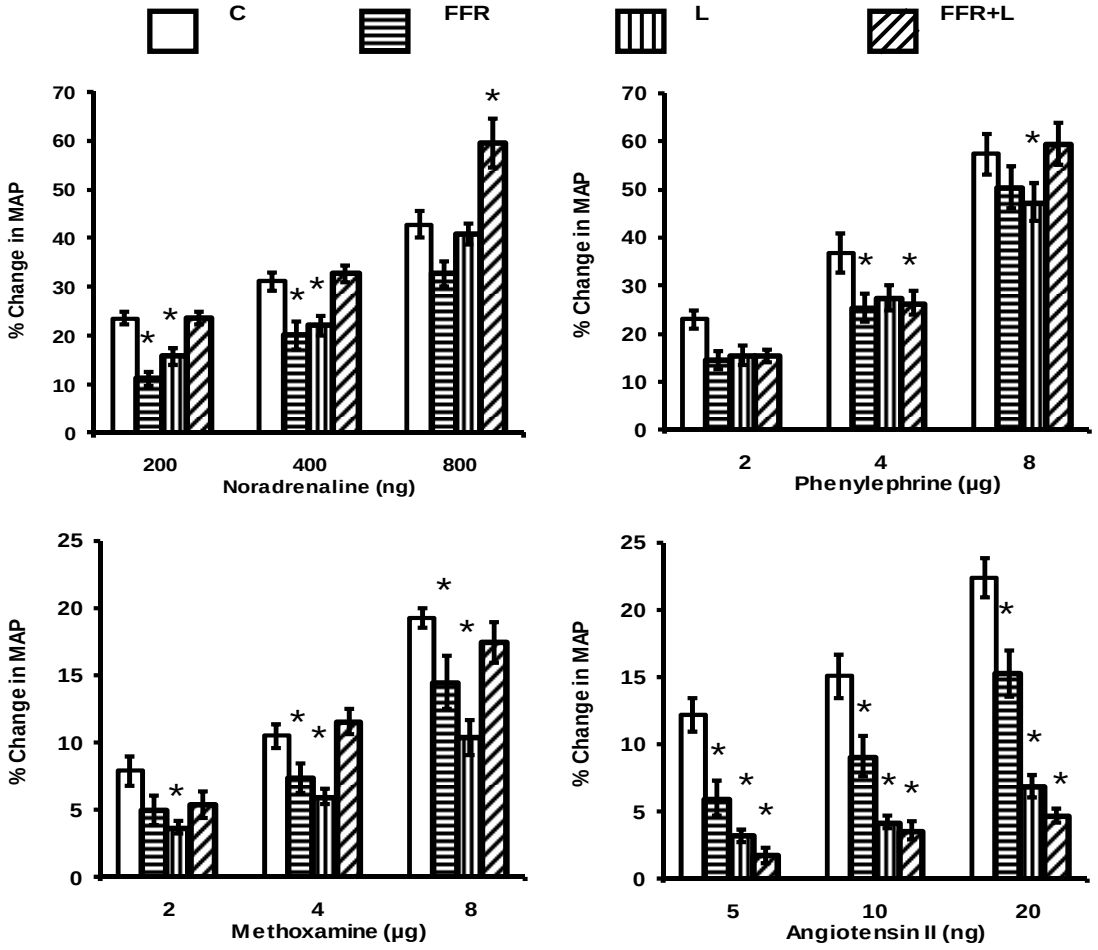

Fig. 1: Vasopressor response to adrenergic agonists and Ang II.
Vasopressor response (expressed as (%) increase in MAP) to graded doses of noradrenaline, phenylephrine, methoxamine and angiotensin II in control (C), fructose-fed (FFR), losartan treated (L) and fructose-fed losartan treated (FFR-L) rats. Data presented as mean±SEM (n = 6). * indicates $p<0.05$ vs. control.

26±2; PE, 30±3; ME, 7±0.7; Ang II, 5±0.4 vs. *C*: NA, 32±2; PE, 40±3; ME, 13±1; Ang II, 17±1) (fig.2). FFR-L had higher ($p<0.05$) vasopressor responses to NA and ME but lower responses to Ang II compared to FFR (*FFR-L*: NA, 39±3; ME, 11±1; Ang II, 3±0.4 vs. *FFR*: NA, 22±2; ME, 9±1; Ang II, 10±1) (fig.2). The vasopressor response to NA was higher but to Ang II was lower in FFR-L compared to C (*FFR-L*: NA, 39±3; Ang II, 3±0.4 vs. *C*: NA, 32±2; Ang II, 17±1) (fig.2).

DISCUSSION

Impairment of the endothelial function has been suggested as a proposed mechanism that links insulin

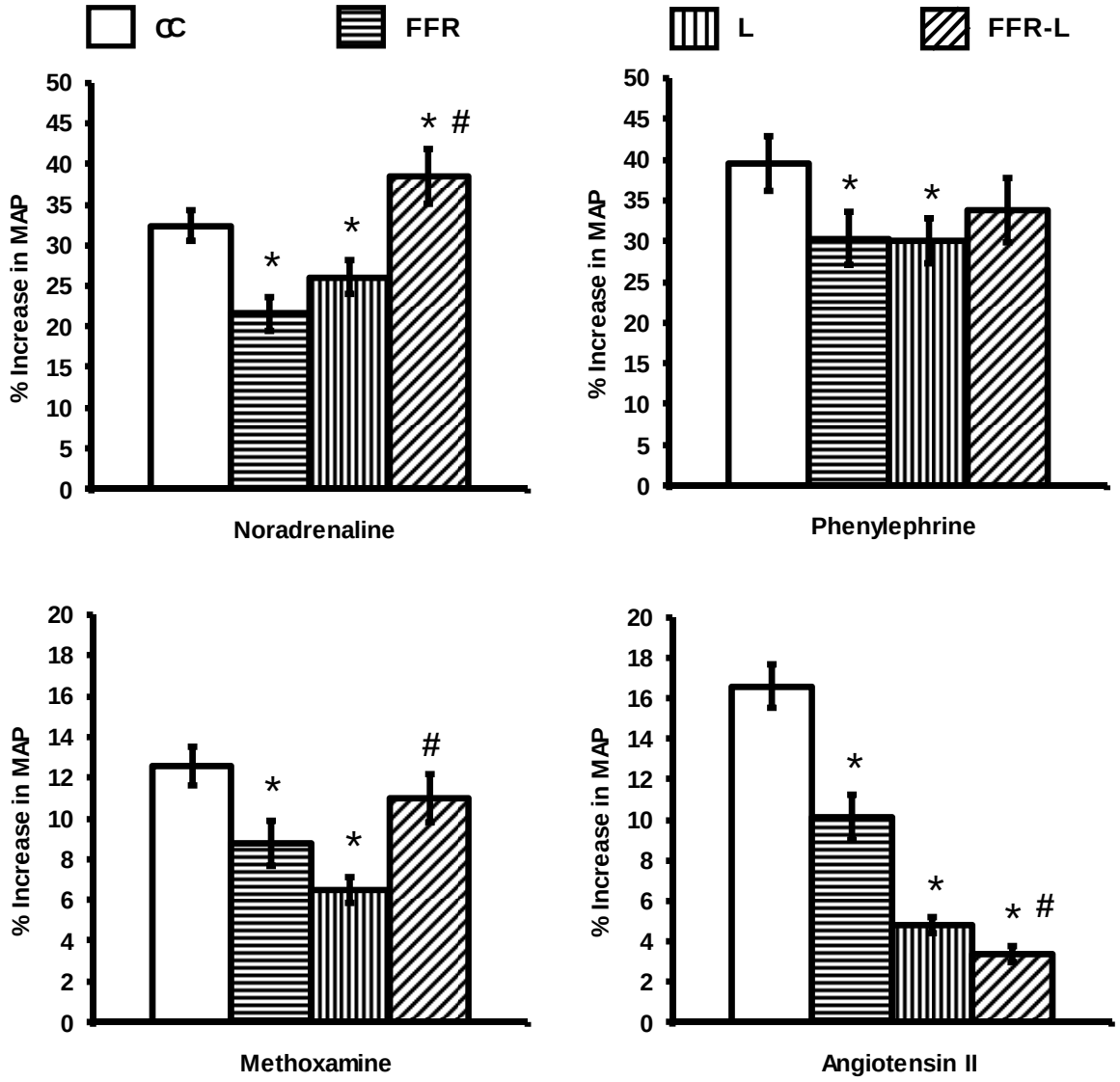

Fig. 2: The overall vasopressor response to adrenergic agonists and Ang II.
Bar graph shows the overall vasopressor response to noradrenaline, phenylephrine, methoxamine and angiotensin II in the systemic vasculature of control (C), fructose-fed (FFR), losartan (L) and fructose-fed rats treated with losartan (FFR-L). Values are mean±SEM of n = 6 rats in each group. $^{*}p<0.05$ vs. respective control. $^{\#}p<0.05$ between FFR-L and FFR.

resistance and high blood pressure in metabolic syndrome (Miller *et al*., 1998). The effect of high and long-term fructose feeding on vascular responses to vasoactive stimuli is the subject of much interest; however, it is still not well understood. The current study showed that fructose feeding for 8 weeks results in hyperinsulinaemia, hyperglycemia, hypertriglyceridaemia and insulin resistance in agreement with a previous report from this laboratory (Abdulla *et al*., 2011). The important finding in the current study is the blunted vasopressor responses of the systemic vasculature to Ang II and adrenergic agonists in the fructose-fed rats, which is supported by our recent findings in the renal vasculature of these rats (Abdulla *et al*., 2012). Impaired vascular responses to exogenous noradrenaline were reported in fructose-fed rats both *in vivo* and *in vitro* (Berger *et al*., 1998, Bunnag *et al*., 1997). The enhanced sympathetic activity in this model of metabolic syndrome might be the cause of this impairment due to an adaptation of the vasculature to its effect, or as a compensatory mechanism in response to structural or functional alterations (Bunnag *et al*., 1997). It is further suggested in an *in vitro* study by Kamata *et al*., (2001) that in fructose-fed mice, there is an impairment of endothelial function in addition to decreased contractile response to α-adrenoceptor agonists. This attenuation in the contractile response is related to an increase in nitric oxide formation which is mediated by endothelial GTP-binding-coupled α_2-adrenoceptors (Kamata *et al*., 2001). The present study suggests that the lower vasopressor response to adrenergic agonists in this

study was the result of high sympathetic activity in these rats. Moreover, higher circulating level of Ang II which is reported in this model (Shinozaki *et al*., 2004, Iyer *et al*., 1996, Giacchetti *et al*., 2000) might be responsible for the altered vascular activity and hence a blunted response to exogenous Ang II.

This study showed that losartan treatment attenuates the vasopressor response to adrenergic agonists. The interactions between RAS and sympathetic nervous system (SNS) have been established at different levels (Zimmerman *et al*., 1984, DiBona, 2000, Abdulla *et al*., 2009b) and suggested to have prominent patho-physiological implications. Ang II is known to facilitate sympathetic neurotransmission at various sites, including the central nervous system (Reid, 1992), sympathetic ganglia (Hosein and Proulx, 1965, Ma *et al*., 2004), and presynaptic sympathetic nerve terminals (Boadle *et al*., 1969, Fabiani *et al*., 2001). Interestingly, vascular AT_1-receptors and α_1-adrenoceptors positively interact with each other and produce an enhanced response to their respective substrate (Vittorio *et al*., 2003). The possible explanation of this interaction is that both receptors may activate common signal systems at the molecular level. Moreover, in rat vascular smooth muscle cells, Ang II is suggested to induce transcription and expression of α_1-adrenoceptors (Hu *et al*., 1995).

AT_1-receptor blockers have been shown to exert beneficial effects on fructose induced metabolic syndrome whether on high blood pressure or insulin resistance (Navarro-Cid *et al*., 1995, Iyer and Katovich, 1996, Iyer and Katovich, 1994). In the present study, although blood pressure of fructose-fed rats measured during the acute study was not significantly higher than the control, however, losartan treatment brought back the systemic haemodynamic values to approach there control values or even lower. Most importantly, altered vascular reactivity was suggested as an effect of AT_1-receptor blockers in this model (Navarro-Cid *et al*., 1995, Shinozaki *et al*., 2004). Furthermore, the activity of endothelial nitric oxide synthase (eNOS) in vascular smooth muscle cells from fructose-fed rats was found to be restored after chronic blockade of AT_1-receptors (Miatello *et al*., 2003). The current study revealed that a 7-day treatment with losartan has restored the vasopressor responses to adrenergic agonists that were blunted due to chronic fructose feeding. The mechanism that is underlying such effect is not known, however, it has been suggested that circulating Ang II is playing a pivotal role in altered haemodynamics in this model (Tran *et al*., 2009). In addition, the dependence of the vascular responses to vasoactive agonists in this study on an intact RAS in fructose-fed rats indicates an important interaction between AT_1-receptors and α_1-adrenoceptors in the systemic vasculature in this model.

In conclusion, this study highlighted the following: First, angiotensin II plays an important role in the control of systemic haemodynamics in fructose-fed rats. Second, an interactive relationship exists between Ang II and adrenergic neurotransmission in the systemic vasculature of fructose-fed rats.

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