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ORIGINAL CONTRIBUTION

The contribution of α_{1B}-adrenoceptor subtype in the renal vasculature of fructose-fed Sprague–Dawley rats

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Abstract
Purpose Fructose feeding induces a moderate increase in blood pressure, insulin resistance, and hyperinsulinemia. This study investigated the role of α_{1B}-adrenoceptor subtype in the control of renal hemodynamic responses to exogenously administered angiotensin II (Ang II) and a set of adrenergic agonists in a model of high fructose-fed rats.
Methods Sprague–Dawley rats were fed for 8 weeks with 20% fructose in drinking water (FFR). The renal cortical vasoconstriction to noradrenaline (NA), phenylephrine (PE), methoxamine (ME) and Ang II in the presence and absence of chloroethylclonidine (CEC) (α_{1B}-adrenoceptor antagonist) was determined. Data, mean ± SEM or SD were subjected to ANOVA with significance at $p < .05$.
Results FFR showed significant increase in the systolic blood pressure, plasma glucose, and insulin levels when compared to control. FFR expressed reduced renal cortical vascular sensitivity to NA, PE, ME, and Ang II. Furthermore, renal cortical vasoconstriction response to NA, PE, ME, and Ang II was blunted in the presence of CEC in control. While in FFR, renal cortical vasoconstriction to NA, PE, and ME was enhanced by CEC. Renal cortical vasoconstriction to Ang II in FFR was reduced in the presence of CEC.
Conclusions In the presence of a hyperinsulinemic state resulting from chronic and high fructose feeding, an attenuated AT_1 and α_1-adrenoceptors response to Ang II and adrenergic stimuli respectively, is expected. In addition, α_{1B}-adrenoceptor is the functional subtype that mediates renal cortical vasoconstriction in control rat, while high fructose feeding did influence the functionality of α_{1B}-adrenoceptor in mediating the renal cortical hemodynamic changes.

Keywords Fructose · Sprague–Dawley rats · Noradrenaline · Hemodynamics · Chloroethylclonidine

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Introduction

Metabolic syndrome is a pathophysiological entity characterized by insulin resistance, hyperinsulinemia, dyslipidemia, hypertension, and obesity [1]. The risk for developing diabetes type 2, cardiovascular disease, and renal disease is increased with increasing manifestations of the various components of the syndrome within any individual. The precise mechanisms behind the development of hypertension in this model have not yet been clearly defined though it has been proposed to be secondary to hyperinsulinemia [2]. In addition, it has been demonstrated that an increase in sympathetic activity could account for the hypertension induced by fructose feeding [3]. Renal α_1-adrenoceptors play important role in the regulation of renal hemodynamic and tubular functions [4]. Pharmacological

and cloning studies have documented, three subtypes of α_1-adrenoceptors: α_{1A}, α_{1B}, and α_{1D} [5, 6]. Among these subtypes, α_{1B}-adrenoceptors have been found to be crucial in the regulation of renal vascular resistance in genetic hypertension and thus contribute to the increased vascular resistance in hypertension [7]. Furthermore, α_{1B}-adrenoceptors are also involved in the mediation of renal tubular sodium and water reabsorption [8]. α_{1B}-Adrenoceptors-mediated renal vasoconstriction has been observed in some pathological conditions in rats, such as diabetes and renal failure [9, 10]. Further, α_{1B}-adrenoceptors gene is expressed in renal microvessels of Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) [7]. Actions of angiotensin II in metabolic syndrome are associated with the development of hypertension, suppression of adiponectin secretion [11], increase of adipocyte size [12], and generation of oxidative stress [13]. It is also known that chronic fructose treatment activates the renin-angiotensin system (RAS), which is manifested by an increase in plasma level of Ang II, elevation of blood pressure, cardiac hypertrophy, and changes in AT_1 receptor density [14]. Although a vast number of studies have investigated the role of renin-angiotensin and sympathetic nervous systems in metabolic syndrome rats, no studies so far investigated the functional contribution of α_{1B}-adrenoceptor subtype in the regulation of renal hemodynamic in rats subjected to high-fructose diet. The present study therefore aimed to investigate the influence of a high fructose intake on the regulation of renal α_1-adrenoceptors-mediated vasoconstriction, with particular attention to the functional contribution of α_{1B}-adrenoceptors in the regulation of renal hemodynamic in SD rats chronically fed on high fructose.

Materials and methods

Animals

Sixteen male Sprague–Dawley (SD) rats with a body weight of 150–180 g were obtained from the Central Animal Facility at Universiti Sains Malaysia, Penang, Malaysia. The rats were permitted a 5-day period-of-acclimatization to the environment, with free access to water and standard rodent chow (Gold Coin Sdn. Bhd., Penang, Malaysia) containing 3% fat, 22% protein and 5% fibers. Thereafter, the rats were randomly assigned to control (C; $n = 8$) that received standard rodent chow, and fructose fed (FFR; $n = 8$) were fed with a standard rodent chow, and fructose was administered as a 20% solution (prepared freshly every day) in drinking water. Both groups were followed for 8 weeks. Experiments were approved by the Ethics Committee of Universiti Sains Malaysia.

Measurements

Body weight, fluid intake, food intake, calorie intake, systolic blood pressure (SBP), non-fasting (NFBG) and fasting (FBG) blood glucose levels, and fasting plasma insulin level of each rat were measured at the end of the treatment period. Food and fluid intakes for each rat were measured by subtracting the amounts remaining in the metabolic cages from the measured quantities provided. Calorie intake was calculated by multiplying the amount of food and fructose consumed each day by the corresponding reference calorie values and summed. Blood samples were collected from the tail at the end of study period on non-fed and fed states. Plasma insulin level was measured in samples collected from rats fasted for 18 h using a quantitative Ultra Sensitive Rat Insulin ELISA kit (Crystal Chem Inc., IL, USA). The reactivity for rat insulin is 100% according to the manufacturer. SBP was measured in conscious rats by tail cuff. All animals were preconditioned for blood pressure measurements 1 week before each experiment. The adiposity [15] and kidney indices [16] were measure by collecting the fat deposit and the left kidney respectively from the rat during the acute experiment at the end of the study. Adiposity index = (Retroperitoneal fat (g) + Epididymal fat (g))/Body weight (g) × 100. Kidney index = Kidney weight (g)/Body weight (g) × 100.

Oral glucose tolerance test (OGTT)

After the 8-week feeding period, the overnight fasted rats were given a 30% (wt/vol) glucose solution at a dose of 3 g/kg body weight by oral gavage [17]. Blood samples (20 μl) were obtained from the tail of conscious rats at baseline and 30, 60, and 120 min after the glucose load. Blood glucose level was determined at each time point using ACCU-CHEK® advantage blood glucose monitoring system (Roche Diagnostics Corporation, Indianapolis, USA). The total area under the curve of glucose response during the OGTT was calculated using the linear trapezoidal rule [18] and used for quantification of the OGTT.

Blood pressure measurements

Blood pressure was recorded using the tail-cuff method in conscious animals as previously reported [18]. Following 3-day training sessions, blood pressure readings were taken from each rat in a calm and dark room, and an average value of ten consecutive systolic blood pressure measurements was calculated. The real measurement started when the rat was stable and the pulse was clear. It is preferable to use readings that are not deviating by more than 5 mmHg.

Animal surgical preparation

Renal vasoconstrictor responses

The in vivo renal vasoconstrictor response experiments were performed as previously mentioned [19, 20]. The overnight fasted rats were anesthetized with 60 mg/kg i.p. sodium pentobarbitone (Nembutal®, CEVA, France). Immediately after induction of anesthesia, the trachea was exposed through a mid-line incision and cannulated. Following tracheostomy, the left carotid artery was cannulated (PE 50, Portex, Kent, UK) and connected to a pressure transducer (model P23 ID Gould, Statham Instruments, UK) attached to a computerized data acquisition system (PowerLab®, ADInstruments, Australia) for the continuous monitoring of mean arterial blood pressure (MAP). The left jugular vein was cannulated (PE 50, Portex, Kent, UK) to infuse a maintenance dose of anesthetic when required. Via a midline abdominal incision, the aorta and the left kidney were exposed. A laser Doppler flowmeter (ADInstruments, Australia) positioned in the outermost layer of the kidney was used to measure renal cortical blood flow (CBF) continuously. The CBF data were further analyzed using a computerized data acquisition and analyzing system (PowerLab®, ADInstruments, Australia). A cannula (PE 50, Portex, Kent, UK) was inserted via the left common iliac artery, such that its beveled tip lay close to the entrance of the renal artery in an attempt to administer noradrenaline (NA), phenylephrine (PE), methoxamine (ME) and angiotensin II (Ang II) close renal arterially [21]. The cannula was kept patent by infusing saline at a rate of 6 ml/kg/h continuously. This cannula is attached to a second pressure transducer (model P23 ID Gould, Statham Instruments, UK) attached to a computerized data acquisition system (PowerLab®, ADInstruments, Australia) for base line measurements of renal arterial pressure (RAP). The urinary bladder was cannulated as well to allow free passage of urine. The animals were allowed at least 1 h to equilibrate after surgery before proceeding to the following steps.

Renal vasoconstrictor response experimental protocol

The renal vasoconstrictor experiments were performed in three phases as follows: after stabilization, base line measurement of MAP, RAP, and CBF was performed. The infusion pump to the iliac artery was stopped before taking the base line RAP value to prevent any interference with the measurements. In Phase I, the animals were infused intrarenal arterially with vehicle (normal saline) at 6 ml/kg/h as control and the first set of renal vasoconstrictor responses to NA, PE, ME, and Ang II was performed, and at least 10 min were left after each agonist to ensure washout. During the second phase, low bolus dose of CEC (5 μg/kg) was injected slowly over 30 s followed by a continuous infusion of CEC (1.25 μg/kg/h) close renal arterially, 15 min later, the second set of renal vasoconstrictor responses to NA, PE, ME, and Ang II was done. In the third and last phase, a high bolus dose of CEC (10 μg/kg) was injected slowly over 30 s followed by a continuous infusion of CEC (2.5 μg/kg/h). Upon reaching the steady state, 15 min later, the same procedure used in the first and second phases was followed [21].

Dose–response curves to NA, PE, ME, and Ang II were undertaken in each phase of the experiment. The administration of agonists was carried out twice in an ascending followed by descending order of doses. Moreover, the agonists were injected in an exchange order every experiment. The doses of the agonists and antagonist were adapted from previous work in this laboratory and intended to produce local action without any significant effect on the systemic blood pressure [20, 21, 22]. The reduction in renal cortical blood flow after bolus doses of NA at 25, 50, 100 and 200 ng, PE at 0.25, 0.50, 1, and 2 μg, ME at 1, 2, 3, and 4 μg and Ang II at 2.5, 5, 10, and 20 ng was determined. The antagonist was given as an initial bolus dose followed by a continuous infusion of one 4th of that dose per hour until completion of the agonist protocol and thereafter the second dose was used (Fig. 1).

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 vasoconstrictor experiment. All drugs were prepared as stock solutions in normal saline on the day of experiment and stored at −4 °C. Noradrenaline is a mixed agonist, and the cardiovascular effects of exogenously administered noradrenaline are mainly characterized by α_1-adrenoceptor-mediated vasoconstriction [23]. Phenylephrine is a non-selective agonist for α_1-adrenoceptors [24], while methoxamine is more selective for α_{1A}-adrenoceptors [25] when compared to α_{1D} adrenoceptor subtype [26]. Angiotensin II is the main peptide of RAS and has a vasoconstrictor action following activation of AT_1 receptors.

Antagonist used

CEC (Research Biochemicals International, USA) is an irreversible antagonist that alkylates and inactivates the function of α_{1B}-adrenoceptors subtype [27]. It also irreversibly inactivates α_{2A}- and α_{2C}-adrenoceptors, whereas α_{1A}- and α_{1B}-adrenoceptor are relatively resistant to its alkylating action [28]. It is used in the present study to

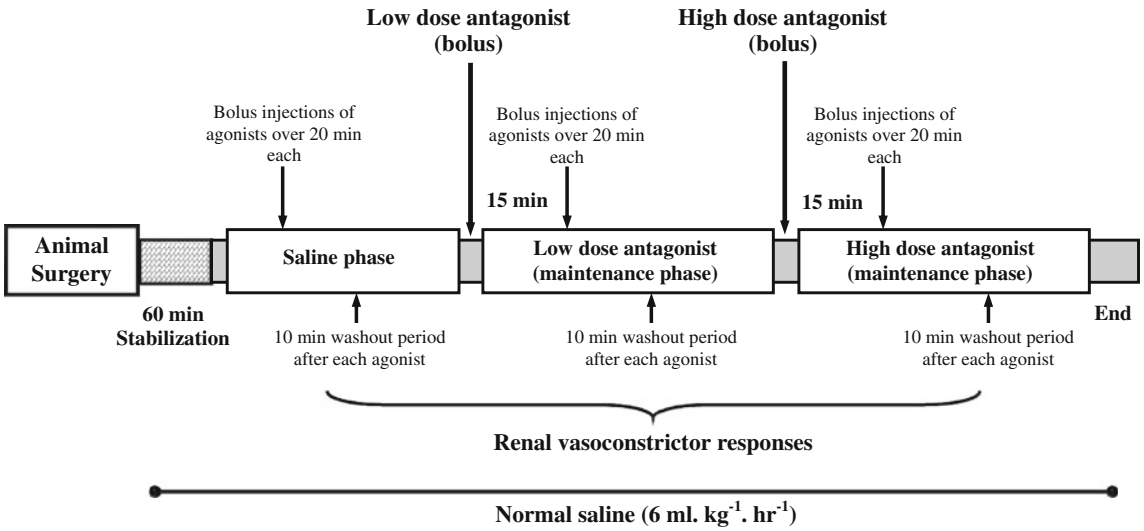

Fig. 1 Three-phase acute renal vasoconstrictor study

characterize the functional contribution of α_{1B}-adrenoceptors in mediating adrenergically induced and angiotensin II-induced renal cortical vasoconstriction in fructose-fed rats.

Statistical analysis

The maximum CBF decrease following each injection of agonist was determined off-line using the software (LabChart 6, ADInstruments, Sydney, Australia). The change in CBF was expressed as percentage of the baseline value. Baseline CBF and MAP were determined from the chart immediately before each agonist injection. The vasoconstrictor responses caused by Ang II and adrenergic agonists were taken as the average values caused by each dose of the agonists administered in ascending and descending orders. The overall mean response for each dose was taken as the average value of the vasoconstrictor responses (drop in CBF) obtained at each dose. The mean values for every phase which are presented in this study (Figs. 2, 3, 4) are the overall mean calculated for all doses of each agonist and compared between high and low dose antagonist phases, and saline phase. All data are expressed as mean ± S.E.M or mean ± SD. The statistical analysis of the data was done by two-way ANOVA followed by Bonferroni post hoc test. While, the analysis of BW, fluid intake, food intake, calorie intake, SBP, NFBG, FBG, plasma insulin level, AUC (OGTT), and base line hemodynamic parameters measured during the acute experiment 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

Metabolic parameters at the end of 8-week feeding period

Metabolic data comparison between FFR and C is shown in Table 1. The result of OGTT that was performed at the end of 8-week feeding period showed that FFR had less tolerance ($p < .05$) to oral glucose load compared to normal rats. Moreover, FFR at the end of treatment period had higher ($p < .05$) values of BW, calorie intake, SBP, FBG, NFBG, and plasma insulin, but lower food intake ($p < .05$) compared to C. However, no significant change in fluid intake was observed between FFR and C at the end of 8-week feeding period. There was no change in kidney index of FFR; however, adiposity index is significantly higher than C.

Base line hemodynamic parameters during the acute experiment

Base line values of MAP, heart rate (HR), CBF, and RAP measured during the control phase (saline) of acute experiment of FFR was not significantly different from C. Moreover, in the second and third phases of acute experiment, no change was seen in base line hemodynamic parameters in FFR or C except for HR compared to their respective control phase (Table 2).

Renal cortical vasoconstrictor response

Adrenergic agonists

NA, PE, and ME produced dose-dependent vasoconstrictions in the control (saline) phase of both C and FFR, as

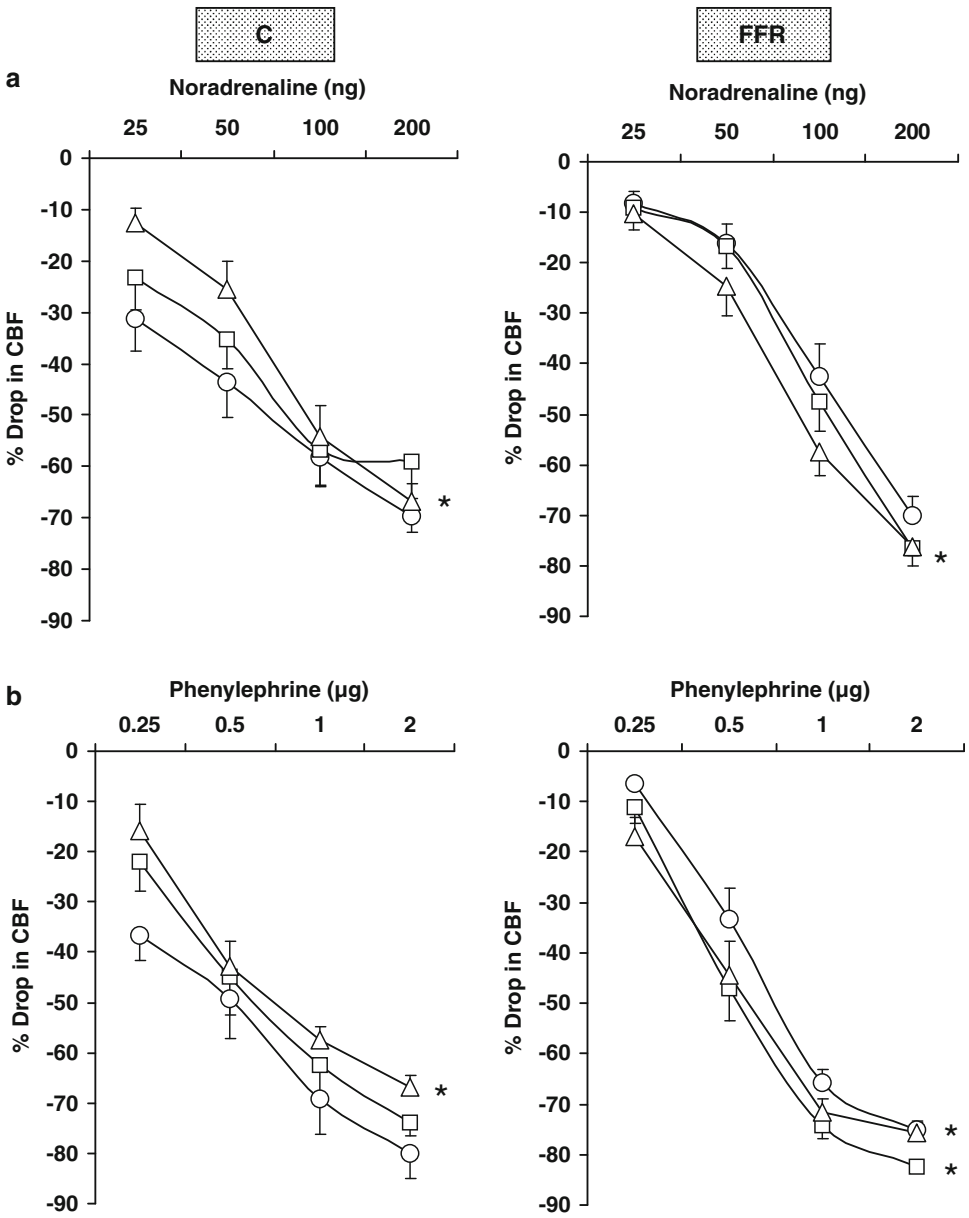

Fig. 2 Line graph shows dose–response curve of the renal vasoconstrictor responses to graded doses of NA (**a**) and PE (**b**) in control and fructose-fed rats during control (saline) phase (*solid line with circle*), low dose of CEC (*solid line with square*) and high dose of CEC (*solid line with triangle*). Values are mean ± SEM of $n = 8$ rats in each group. The significance is between the overall mean of responses due to four doses of agonist during each phase and compared to control phase. * $p < .05$ vs. saline phase

can be seen from the dose–response curves in Figs. 2 and 3. The renal vasoconstriction responses produced by NA, PE, and ME in FFR during the control phase (in absence of antagonist) were significantly lower ($p < .05$) when compared to C (*FFR*: NA, 35 ± 4; PE, 43 ± 5; ME; 25 ± 4 vs. *C*: NA, 51 ± 4; PE, 60 ± 3; ME, 43 ± 4). This is shown either through the dose–response curves (Figs. 2a, b, 3a) or when the overall mean (overall mean of responses for all doses of agonist employed) was shown (Fig. 4). In the later subsequent phases of the experiment, renal vasoconstrictor responses induced by NA, PE, and ME were rather attenuated by both low dose of CEC but significantly ($p < .05$) by high dose of CEC in C relative to their corresponding control (saline) phase (*high-dose CEC phase*: NA, 40 ± 4; PE, 49 ± 4; ME, 33 ± 4 vs. *saline phase*: NA, 51 ± 4; PE, 60 ± 3; ME, 43 ± 4). These effects collectively contributed to a significant reduction in the overall % drop of CBF (Fig. 4). In FFR, NA-, PE-, and ME-induced renal vasoconstriction was enhanced significantly ($p < .05$) by low or high dose of CEC; however, NA response is significantly enhanced with a high dose of CEC only, (*low-dose CEC phase*: NA, 38 ± 4; PE, 54 ± 5; ME, 33 ± 4 vs. *saline phase*: NA, 35 ± 4; PE, 43 ± 5; ME; 25 ± 4) and (*high-dose CEC phase*: NA, 43 ± 4; PE, 51 ± 4; ME, 32 ± 4 vs. *saline phase*: NA, 35 ± 4; PE, 43 ± 5; ME; 25 ± 4) (Fig. 4).

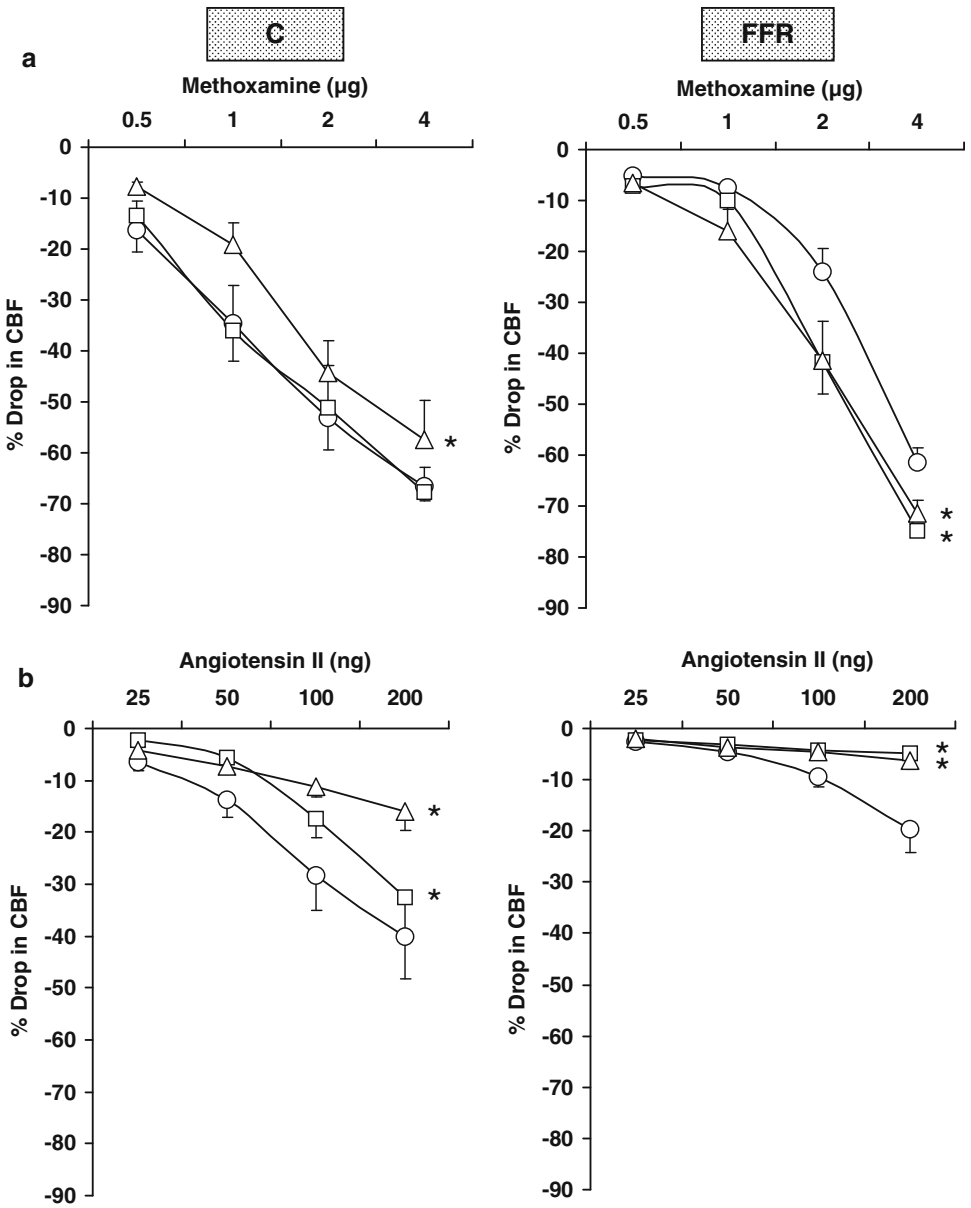

Fig. 3 Line graph shows dose–response curve of the renal vasoconstrictor responses to graded doses of ME (**a**) and Ang II (**b**) in control and fructose-fed rats during control (saline) phase (*solid line with circle*), low dose of CEC (*solid line with square*) and high dose of CEC (*solid line with triangle*). Values are mean ± SEM of $n = 8$ rats in each group. The significance is between the overall mean of responses due to 4 doses of agonist during each phase and compared to control phase. * $p < .05$ vs. saline phase

Angiotensin II

Ang II caused dose-related drop in cortical blood flow in the control phase of both C and FFR (Fig. 3B). As shown for adrenergic agonists, Ang II produced cortical vasoconstrictor response in FFR that is lower ($p < .05$) compared to C (*FFR*: 9 ± 1 vs. *C*: 23 ± 3). Interestingly, in the presence of low or high dose of CEC in either FFR or C, the drop in cortical blood flow due to Ang II was lower ($p < .05$) compared to control (saline) phase. For FFR (*low-dose CEC phase*: 4 ± 0.3 and *high-dose CEC phase*: 4 ± 0.5 vs. *saline phase*: 9 ± 1), while for C (*low-dose CEC phase*: 15 ± 3 and *high-dose CEC phase*: 10 ± 1 vs. *saline phase*: 23 ± 3) (Fig. 4). Therefore, it appears on the contrary to adrenergic stimuli, Ang II in the low- or high-dose antagonist phases of FFR produced reduction in cortical perfusion that is lower compared to saline phase.

The administration of NA, PE, ME, Ang II, and CEC brought no effect on baseline measurements of MAP, CBF, and RAP in all of the three phases of acute vasoconstrictor experiment.

Discussion

Chronic and high fructose consumption by rat in this study produced insulin resistance, elevation in blood pressure, and higher adiposity. This although well known for metabolic syndrome model induced by high-fructose diet, the current study to our knowledge is from few studies to use

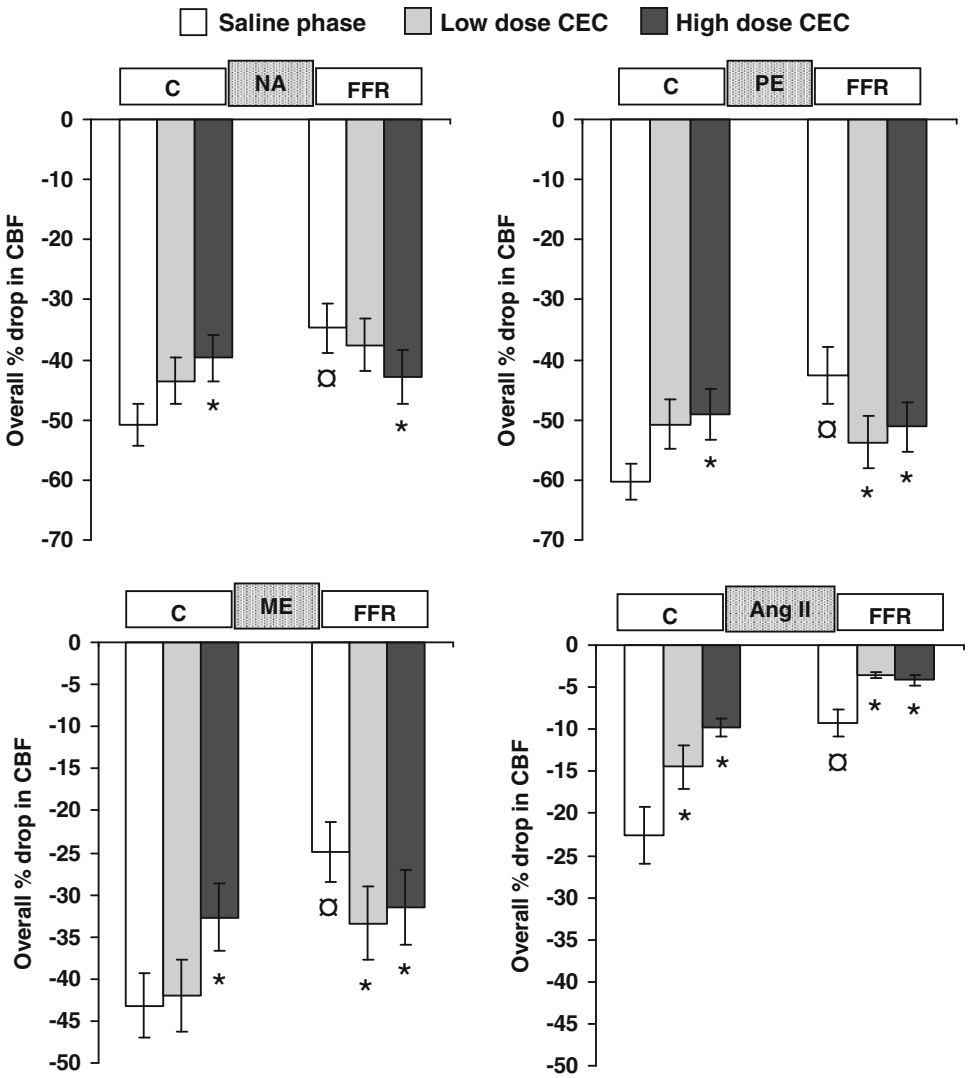

Fig. 4 Bar graph shows the overall % drop in the renal cortical blood flow in response to NA, PE, ME, and Ang II in control and fructose-fed rats in the absence and presence of low and high dose of CEC. The overall mean is the mean of all responses due to 4 doses of each agonist during each phase. Values are mean ± SEM of $n = 8$ rats in each group. ¤ $p < .05$ between FFR and C saline phases. * $p < .05$ vs. saline phase

Table 1 Effect of 8 weeks of fructose feeding on body weight (BW), fluid intake, food intake, calorie intake, systolic blood pressure (SBP), fasting blood glucose (FBG), non-fasting blood glucose (NFBG), plasma insulin level, kidney and adiposity index and AUC (OGTT)

Parameter	*n*	Group	
		C	FFR
B.wt (g)	7	281.3 ± 18.6	312.3 ± 7.1*
Fluid intake (ml/kg/d)	6	139.9 ± 23.4	148.2 ± 33.1
Food intake (g/kg/d)	6	58.4 ± 1.4	46.3 ± 3.9*
Calorie intake (Kcal/kg/d)	6	210.1 ± 22.7	266.7 ± 21.5*
SBP (mmHg)	7	105.0 ± 9.0	143.0 ± 17.0*
FBG (mmol/L)	8	4.1 ± 0.6	5.2 ± 0.9*
NFBG (mmol/L)	8	7.3 ± 1.1	8.8 ± 1.4*
Plasma insulin (ng/ml)	6	2.6 ± 0.5	4.7 ± 1.6*
Kidney index (%)	8	0.33 ± 0.04	0.34 ± 0.03
Adiposity index (%)	8	1.6 ± 0.3	2.9 ± 0.5*
AUC (OGTT) (mmol/L min)	6	664.5 ± 103.5	789.1 ± 56.9*

Values are mean ± SD, * $p < .05$ between FFR and C

high concentration of fructose in drinking water. To the present day, the effects of high fructose feeding on renal vasoconstriction are less clear. Furthermore, the pathogenesis of metabolic syndrome in fructose-fed rats may also depend on a dysfunctional peripheral sympathetic nervous system [29]. In the current study, the magnitude of renal vasoconstrictor responses to angiotensin II and adrenergic agonists in fructose-fed rats is lower than the control. This indeed shows that the high fructose intake by Sprague–Dawley rats in this study had attenuated the sensitivity of the renal cortical vasculature to exogenous Ang II and adrenergic stimuli. This well agrees with the observations by others that in fructose-fed rats, there is impaired sensitivity to noradrenaline with an increase in its urinary excretion [30, 31]. This may have resulted as a compensatory change that follows the enhancement of the activity of the sympathetic nervous system producing receptor desensitization or downregulation [32, 33]. Moreover, the elevation in Ang II level and the altered

Table 2 Base line hemodynamic parameters measured during the acute renal vasoconstrictor experiment

Phase	Group							
	C				FFR			
	MAP (mmHg)	HR (bpm)	CBF (BPU/min)	RAP (mmHg)	MAP (mmHg)	HR (bpm)	CBF (BPU/min)	RAP (mmHg)
Saline phase	116 ± 6	256 ± 11	163 ± 9	112 ± 4	119 ± 4	311 ± 6	168 ± 9	121 ± 3
Low dose CEC	121 ± 7	229 ± 12	127 ± 10	108 ± 6	109 ± 3	238 ± 7*	161 ± 10	110 ± 4
High dose CEC	120 ± 7	200 ± 26	122 ± 7	107 ± 7	110 ± 2	204 ± 11*	137 ± 14	108 ± 2

Values are mean ± SEM of n = 6-8 rats in each group. $^{*}p < .05$ vs. saline phase. MAP, mean arterial blood pressure; HR, heart rate; CBF, cortical blood flow; RAP, renal arterial pressure, bpm, beat per minute; BPU, blood perfusion unit

tissue AT_1 receptors in high fructose–fed rats is extensively highlighted [13, 34, 35]. In an in vitro study, thoracic aorta showed early exaggerated responses to phenylephrine and Ang II that were not observed after a longer period of fructose feeding [13, 36]. The present study thus suggested that high fructose feeding attenuates the sensitivity of the renal cortical vasculature to exogenous Ang II and α_1-adrenergic agonists which is obvious from blunted renal cortical vasoconstriction to exogenous agonists.

The interaction in the renal vasculature between AT_1 receptors and α_1-adrenoceptors has recently being the subject of much interest. Evidence has been provided on the cross talk between α_1-adrenoceptors and Ang II receptors in smooth muscle of rabbit aorta [37]. Moreover, several in vivo studies have indicated that blocking of α_1-adrenoceptors attenuates renal vascular response to Ang II in rat and rabbit [20, 22, 38, 39]. Relevant physiological interactions at both cellular and molecular levels between the AT_1 and adrenoceptors have been described, since both receptors activate common signal systems. It is suggested that released noradrenaline in the neurons interacts with α_1-adrenoceptors and their chronic stimulation in turn results in down regulation of AT_1-receptors [40]. Moreover, Ang II induces transcription and expression of α_1-adrenoceptors in rat vascular smooth muscle cells [41]. The renal vasoconstrictor responses to adrenergic agonists in this study were blunted following the administration of CEC as expected; however, interestingly and in agreement with previous observations, there was a marked reduction as well in the renal cortical response to Ang II in control or fructose-fed rats. A cross-talk relationship between AT_1 receptors and α_{1B}-adrenoceptors is suggested to play a pivotal role in modulating the vascular response in this model.

Further we observed that NA, PE, and ME-induced renal cortical vasoconstrictions were further enhanced by both low and high dose of CEC in the second and third phase compared to the corresponding control phase in fructose-fed rats. The exact mechanism underlying this type of response is not known yet; however, there is a possibility that the CEC antagonism of postsynaptic α_{1B}-adrenoceptors interacts and enhances sensitivity of other α_1-adrenoceptor subtypes to exogenous NA, PE, and ME [10, 24] resulting in a larger postsynaptic response in the renal cortical vasculature of fructose-fed rats. It is therefore an interactive relationship between the α_1-adrenoceptor subtypes. Such interaction is reported to exist in the cardiovascular system of rat [42]. In that it is reported that α_{1B}-adrenoceptors inhibit the activities of α_{1A}-adrenoceptors in neonatal rat myocardium so that the inactivation of α_{1B}-adrenoceptors by CEC potentiated the effects of nonselective α_1-adrenoceptors agonist phenylephrine on myocardial protein synthesis and gene expression [42]. The same pattern of results by which CEC-mediated augmentation of renal vasoconstrictor response to adrenergic agonists has also been reported in other pathophysiological state, such as in rats with renal impairment and in high-sodium diet-fed rats [10, 43]. It is noteworthy to mention that in agreement with a previous study in rat myocardium [42], altered renal vascular response to adrenergic agonists in this study after treatment with CEC is due to primarily the inactivation of α_{1B}-adrenoceptors but not due to inactivation of α_{1A}- and α_{1D}-adrenoceptors. Further studies are required to clarify in a more mechanistic way the reason behind that. In addition, there was a reduction in the renal cortical blood perfusion in the second and third phase of fructose-fed rats or control. This reduction is not achieving significance even in time control experiments (data not shown) in which only saline was infused throughout the experiment.

According to Deng et al. [44], in an in vitro study, the inactivation of α_{1B}-adrenoceptors by CEC which potentiates the effect of α_1-adrenergic agonists is due to increasing Ca^{2+} current. Furthermore, phenylephrine resulted in a translocation of Ca^{2+}-independent protein kinase C (PKC) isoforms δ and ε but produced little effect on Ca^{2+}-dependent PKCα and on PKCζ as previously mentioned [44]. However, after the inactivation of α_{1B}-adrenoceptors by CEC, phenylephrine causes translocation of Ca^{2+}-dependent PKCα isoform markedly. This is due to

α_{1B}-adrenoceptors activation that inhibits the translocation of PKCα; however, its inactivation is necessary for its translocation by α_1-adrenoceptors agonist [42]. Our study showed an enhancement in the renal vascular responses to phenylephrine, methoxamine, and noradrenaline that is in the fructose-induced metabolic syndrome but not in the control state following the infusion of CEC. Our current data is not supported with studies at the molecular level to strengthen further the suggestion of an interaction between α_1-adrenoceptors subtypes which is one of the limitations at this level; however, based on hemodynamic data presented here, the current study clearly showed the contribution of α_{1B}-adrenoceptor subtype in the renal cortical vasculature of fructose-fed rats.

Taken together, data of this study reveal an interesting interaction between α_{1B}-adrenoceptor subtype and AT_1 receptors in the renal vasculature of fructose-induced metabolic syndrome. Further, to our knowledge, this is the first study to investigate the effect of high fructose intake on the renal vascular α_1-adrenoceptor subtypes' activity and more specifically on α_{1B}-adrenoceptors. The involvement of α_{1B}-adrenoceptors in renal vasoconstriction of fructose-fed rats is concurrent with the results obtained from recent studies of other forms of hypertension such as rats subjected to high-sodium diet and DOCA-salt hypertensive rats [43, 45]. Moreover, this also has been reported in other pathological conditions such as heart failure [46], diabetes, and renal failure [9, 10, 24].

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References

1. Reaven GM (1997) Banting Lecture 1988. Role of insulin resistance in human disease. 1988. Nutrition 13(1):65
2. Navarro-Cid J, Maeso R, Perez-Vizcaino F, Cachofeiro V, Ruilope LM, Tamargo J, Lahera V (1995) Effects of losartan on blood pressure, metabolic alterations, and vascular reactivity in the fructose-induced hypertensive rat. Hypertension 26(6 Pt 2): 1074–1078
3. Hwang IS, Ho H, Hoffman BB, Reaven GM (1987) Fructose-induced insulin resistance and hypertension in rats. Hypertension 10(5):512–516
4. DiBona GF, Kopp UC (1997) Neural control of renal function. Physiol Rev 77(1):75–197
5. Garcia-Sainz JA, Vazquez-Prado J, del Carmen Medina L (2000) Alpha 1-adrenoceptors: function and phosphorylation. Eur J Pharmacol 389(1):1–12
6. Salomonsson M, Brannstrom K, Arendshorst WJ (2000) Alpha(1)-adrenoceptor subtypes in rat renal resistance vessels: in vivo and in vitro studies. Am J Physiol Renal Physiol 278(1):F138–147
7. Canessa LM, Piccio MM, Vachvanichsanong P, Sidhu A, Porter CC, Robillard JE, Felder RA, Jose PA (1995) Alpha 1B-adrenergic receptors in rat renal microvessels. Kidney Int 48(5):1412–1419
8. Elhawary AM, Pang CC (1994) Alpha 1b-adrenoceptors mediate renal tubular sodium and water reabsorption in the rat. Br J Pharmacol 111(3):819–824
9. Khan MA, Sattar MA, Abdullah NA, Abdulla MH, Salman IM, Kazi RN, Swarup KR, Rathore HA, Basri F, Hussain NM, Dewa A, Johns EJ (2009) Functional subtypes of renal alpha1-adrenoceptor in spontaneously hypertensive rats with streptozotocin-induced experimental diabetic nephropathy. Kidney Blood Press Res 32(5):349–359
10. Khan MA, Sattar MA, Abdullah NA, Johns EJ (2008) Alpha1B-adrenoceptors mediate adrenergically-induced renal vasoconstrictions in rats with renal impairment. Acta Pharmacol Sin 29(2):193–203
11. Ran J, Hirano T, Fukui T, Saito K, Kageyama H, Okada K, Adachi M (2006) Angiotensin II infusion decreases plasma adiponectin level via its type 1 receptor in rats: an implication for hypertension-related insulin resistance. Metabolism 55(4):478–488
12. Furuhashi M, Ura N, Takizawa H, Yoshida D, Moniwa N, Murakami H, Higashiura K, Shimamoto K (2004) Blockade of the renin-angiotensin system decreases adipocyte size with improvement in insulin sensitivity. J Hypertens 22(10):1977–1982
13. Shinozaki K, Ayajiki K, Nishio Y, Sugaya T, Kashiwagi A, Okamura T (2004) Evidence for a causal role of the renin-angiotensin system in vascular dysfunction associated with insulin resistance. Hypertension 43(2):255–262
14. Iyer SN, Katovich MJ, Raizada MK (1996) Changes in angiotensin AT1 receptor density during hypertension in fructose-fed rats. Adv Exp Med Biol 396:49–58
15. Taylor BA, Phillips SJ (1996) Detection of obesity QTLs on mouse chromosomes 1 and 7 by selective DNA pooling. Genomics 34(3):389–398
16. Morioka Y, Koike H, Ikezumi Y, Ito Y, Oyanagi A, Gejyo F, Shimizu F, Kawachi H (2001) Podocyte injuries exacerbate mesangial proliferative glomerulonephritis. Kidney Int 60(6): 2192–2204
17. Li YQ, Ji H, Zhang YH, Ding DY, Ye XL (2006) Metabolic effects of telmisartan in spontaneously hypertensive rats. Naunyn Schmiedebergs Arch Pharmacol 373(4):264–270
18. Abdullah MM, Xu Z, Pierce GN, Moghadasian MH (2007) The effects of simultaneous administration of dietary conjugated linoleic acid and telmisartan on cardiovascular risks in rats. Lipids 42(9):855–864
19. Just A, Olson AJ, Whitten CL, Arendshorst WJ (2007) Superoxide mediates acute renal vasoconstriction produced by angiotensin II and catecholamines by a mechanism independent of nitric oxide. Am J Physiol Heart Circ Physiol 292(1): H83–92
20. Abdulla MH, Sattar MA, Abdullah NA, Khan MA, Abdallah HH, Johns EJ (2009) Chronic treatment with losartan and carvedilol differentially modulates renal vascular responses to sympathomimetics compared to treatment with individual agents in normal Wistar Kyoto and spontaneously hypertensive rats. Eur J Pharmacol 612(1–3):69–74
21. Hye Khan MA, Sattar MA, Abdullah NA, Johns EJ (2008) Influence of combined hypertension and renal failure on functional alpha(1)-adrenoceptor subtypes in the rat kidney. Br J Pharmacol 153(6):1232–1241
22. Abdulla MH, Sattar MA, Khan MA, Abdullah NA, Johns EJ (2009) Influence of sympathetic and AT-receptor blockade on angiotensin II and adrenergic agonist-induced renal vasoconstrictions in spontaneously hypertensive rats. Acta Physiol (Oxf) 195(3):397–404

23. Schafers RF, Poller U, Ponicke K, Geissler M, Daul AE, Michel MC, Brodde OE (1997) Influence of adrenoceptor and muscarinic receptor blockade on the cardiovascular effects of exogenous noradrenaline and of endogenous noradrenaline released by infused tyramine. Naunyn Schmiedebergs Arch Pharmacol 355(2):239–249
24. Armenia A, Munavvar AS, Abdullah NA, Helmi A, Johns EJ (2004) The contribution of adrenoceptor subtype(s) in the renal vasculature of diabetic spontaneously hypertensive rats. Br J Pharmacol 142(4):719–726
25. Tsujimoto G, Tsujimoto A, Suzuki E, Hashimoto K (1989) Glycogen phosphorylase activation by two different alpha 1-adrenergic receptor subtypes: methoxamine selectively stimulates a putative alpha 1-adrenergic receptor subtype (alpha 1a) that couples with Ca2 + influx. Mol Pharmacol 36(1):166–176
26. Arevalo-Leon LE, Gallardo-Ortiz IA, Urquiza-Marin H, Villalobos-Molina R (2003) Evidence for the role of alpha1D- and alpha1A-adrenoceptors in contraction of the rat mesenteric artery. Vascul Pharmacol 40(2):91–96
27. Nunes JP, Guimaraes S (1993) Chloroethylclonidine irreversibly activates postjunctional alpha 2-adrenoceptors in the dog saphenous vein. Naunyn Schmiedebergs Arch Pharmacol 348(3): 264–268
28. Michel MC, Kerker J, Branchek TA, Forray C (1993) Selective irreversible binding of chloroethylclonidine at alpha 1- and alpha 2-adrenoceptor subtypes. Mol Pharmacol 44(6):1165–1170
29. Tran LT, Yuen VG, McNeill JH (2009) The fructose-fed rat: a review on the mechanisms of fructose-induced insulin resistance and hypertension. Mol Cell Biochem 332(1–2):145–159
30. Bunnag P, Hori MT, Ormsby B, Berger ME, Golub MS, Tuck ML (1997) Impaired in vivo adrenergic responses in diet-induced hypertensive rats. Hypertens Res 20(1):17–21
31. Kamide K, Rakugi H, Higaki J, Okamura A, Nagai M, Moriguchi K, Ohishi M, Satoh N, Tuck ML, Ogihara T (2002) The renin-angiotensin and adrenergic nervous system in cardiac hypertrophy in fructose-fed rats. Am J Hypertens 15(1 Pt 1):66–71
32. Sun CL, Hanig JP (1983) Vascular reactivity to adrenergic agents and neuronal and vascular catecholamine levels in spontaneously hypertensive rats. Pharmacology 27(6):319–324
33. Hogikyan RV, Supiano MA (1994) Arterial alpha-adrenergic responsiveness is decreased and SNS activity is increased in older humans. Am J Physiol 266(5 Pt 1):E717–E724
34. Iyer SN, Raizada MK, Katovich MJ (1996) AT1 receptor density changes during development of hypertension in hyperinsulinemic rats. Clin Exp Hypertens 18(6):793–810
35. Nyby MD, Abedi K, Smutko V, Eslami P, Tuck ML (2007) Vascular Angiotensin type 1 receptor expression is associated with vascular dysfunction, oxidative stress and inflammation in fructose-fed rats. Hypertens Res 30(5):451–457
36. Iyer SN, Katovich MJ (1996) Vascular reactivity to phenylephrine and angiotensin II in hypertensive rats associated with insulin resistance. Clin Exp Hypertens 18(2):227–242
37. Jerez S, Peral de Bruno M, Coviello A (2004) Cross talk between angiotensin II and alpha 1 adrenergic receptors in rabbit aorta: role of endothelium. J Cardiovasc Pharmacol 43(3):402–409
38. Abdulla MH, Sattar MA, Abdullah NA, Hazim AI, Anand Swarup KR, Rathore HA, Khan MA, Johns EJ (2008) Inhibition of Ang II and renal sympathetic nerve influence dopamine-and isoprenaline-induced renal haemodynamic changes in normal Wistar-Kyoto and spontaneously hypertensive rats. Auton Autacoid Pharmacol 28(4):95–101
39. Chen K, Zimmerman BG (1995) Angiotensin II-mediated renal vasoconstriction amenable to alpha 1-adrenoceptor blockade. Eur J Pharmacol 284(3):281–288
40. Sumners C, Raizada MK (1993) Angiotensin II receptor subtypes in neuronal cells. In: Raizada MK, Phillips MI, Sumners C (eds) Cellular and molecular biology of the renin-angiotensin systemed. CRC, Boca Raton, FL, pp 379–411
41. Hu ZW, Shi XY, Okazaki M, Hoffman BB (1995) Angiotensin II induces transcription and expression of alpha 1-adrenergic receptors in vascular smooth muscle cells. Am J Physiol 268(3 Pt 2): H1006–H1014
42. Deng XF, Sculptoreanu A, Mulay S, Peri KG, Li JF, Zheng WH, Chemtob S, Varma DR (1998) Crosstalk between alpha-1A and alpha-1B adrenoceptors in neonatal rat myocardium: implications in cardiac hypertrophy. J Pharmacol Exp Ther 286(1):489–496
43. Kazi RN, Munavvar AS, Abdullah NA, Khan AH, Johns EJ (2009) Influence of high dietary sodium intake on the functional subtypes of alpha-adrenoceptors in the renal cortical vasculature of Wistar-Kyoto rats. Auton Autacoid Pharmacol 29(1–2):25–31
44. Deng XF, Mulay S, Varma (1997) Role of Ca(2+)-independent PKC in alpha 1-adrenoceptor-mediated inotropic responses of neonatal rat hearts. Am J Physiol 273(3 Pt 2):H1113–H1118
45. Perez-Rivera AA, Fink GD, Galligan JJ (2005) Alpha-1B adrenoceptors mediate neurogenic constriction in mesenteric arteries of normotensive and DOCA-salt hypertensive mice. Auton Neurosci 121(1–2):64–73
46. Abbas SA, Munavvar AS, Abdullah NA, Johns EJ (2006) Involvement of α_1-adrenoceptor subtypes in the cardiac failure in Spontaneously Hypertensive rats. J Basic Appl Sci 2:59–69