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Impaired hypotensive responses induced by intrathecally injected drugs in fructose-fed rats

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ABSTRACT

Blood pressure responses to intrathecal (*i.t.*) injection of neurochemicals were examined in the fructose-fed rat, an experimental model of metabolic syndrome.Sprague-Dawley rats receiving either tap water or water containing 10% fructose during 8 weeks were anesthetized with sodium pentobarbital. The endocannabinoid anandamide (100 nmol; *i.t.*) decreased mean blood pressure in control rats (-21.2 ± 6.3 mmHg), but had no effect in fructose-fed animals. Similarly, calcitonin gene-related peptide (CGRP; 0.125 nmol; *i.t.*) decreased mean blood pressure in control, but not in treated rats. The high fructose diet did not cause significant changes in the pressor effects of *i.t.* administered noradrenaline (100 nmol) and *N*-methyl-D-aspartate (30 nmol). The nitric oxide donor sodium nitroprusside (500 nmol, *i.t.*) induced a brief hypotension followed by a sustained increase in mean blood pressure in control rats; however, this drug only produced pressor effects in fructose-fed animals. The $GABA_A$-receptor agonist muscimol (8.8 nmol, *i.t.*) and the $GABA_B$-receptor agonist baclofen (100 nmol, *i.t.*) decreased mean blood pressure 30–35 mmHg, both in control and in fructose-fed rats. Fructose potentiated the pressor effect of *i.v.* injected noradrenaline, but did not modify the hypotensive responses to *i.v.* administered sodium nitroprusside and acetylcholine.These results could suggest that, in pentobarbital-anesthetized rats, fructose feeding could alter spinal mechanisms of regulation of preganglionic sympathetic nerve activity. It is proposed that the spinal cord could be involved in the sympathetic dysfunction associated with the metabolic syndrome.

1. Introduction

The metabolic syndrome is a combination of cardiovascular risk factors such as high blood pressure, hyperglycemia, hypertriglyceridemia and obesity. These features, often accompanied by hyperinsulinemia and insulin resistance, may evolve into type 2 diabetes and myocardial infarction (Reaven, 2002; Basciano et al., 2005; Després et al., 2008; Deedwania, 2011).

The metabolic syndrome is closely related to autonomic nervous system dysfunction. In this regard, increased sympathetic nerve activity was observed in humans diagnosed with the syndrome (Mancia et al., 2007; Grassi et al., 2009). Evidence of an increased sympathetic drive was also reported for fructose-fed mice, an experimental model of the disease (Farah et al., 2006). Furthermore, it was reported that the hypertensive state and insulin resistance evidenced in fructose-fed rats are associated with changes in the interaction between angiotensinergic and noradrenergic nerve pathways in the hypothalamus, a major sympathetic nervous system regulatory center (Mayer et al., 2006, 2008). Moreover, it was found that obesity-induced hypertension, a condition often observed in patients with metabolic syndrome, could be related to sympatho excitation caused by an increase in oxidative stress in cardiovascular regulatory centers such as hypothalamic nuclei and the rostral ventrolateral medulla (Nagae et al., 2009; Kishi et al., 2011).

The preganglionic sympathetic neurons in the spinal cord are the last relay station in the regulation of sympathetic outflow by the central nervous system. This is accomplished through the effects of several neurotransmitters and neuromodulators that are released in the spinal cord from either bulbospinal pathways or interneurons (Coote, 1988; Tang et al., 2004; Llewellyn-Smith, 2009). In line with this, our previous studies showed that intrathecal (*i.t.*) injection of excitatory amino acids and high doses of catecholamines induce pressor responses, whereas calcitonin gene related peptide (CGRP), the endocannabinoid anandamide and low doses of catecholamines, administered in the thoracolumbar spinal cord, induce hypotensive responses in anesthetized rats (García et al., 1996, 2006; Celuch and García, 2002). We also reported that *i.t.* injection of the nitric oxide donor sodium nitroprusside produced a biphasic response that consisted of hypotension followed by hypertension (García et al., 1998).

Taking into account that the metabolic syndrome is associated to sympathetic dysfunction, and that several neurotransmitters

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and neuromodulators in the spinal cord are involved in the regulation of the sympathetic outflow, the aim of the present study was to examine whether there were changes in blood pressure responses induced by intrathecally injected neurochemicals in rats receiving 10% fructose in the drinking water during 8 weeks.

2. Materials and methods

2.1. Animals and fructose treatment

This study was performed in accordance with the guide for the Care and Use of Laboratory Animals of the National Research Council (USA, 1996). It did not imply actual or potential conflicts of financial, personal and institutional interests.

Male Sprague-Dawley rats (220–240 g at the beginning of treatment) were maintained in a room with controlled temperature (19–22 °C) and a 12-h light–dark cycle. The animals were randomly divided into two groups: control rats received standard chow and tap water, whereas fructose-fed animals received standard chow and 10% (w/v) fructose in the drinking water during 8 weeks.

The daily intake of water was measured at the 8th week of treatment. The average of the total drinking volume for two animals housed together in the same cage was considered as a single data.

2.2. Systolic blood pressure in conscious rats

Systolic blood pressure was measured in the 8th week at midday, by tail-cuff plethysmography, using a microphone and an occlusion cuff (Kent Scientific Corporation, USA) connected to an EKG-pulse sphygmomanometer (Grass, model 7P8F). Animals were trained in the procedure for three days at the 7th week of treatment. Systolic blood pressure value for each rat was the mean of at least 5 consecutive readings.

2.3. Biochemical determinations in overnight fasting animals

Glycemia was measured with an OneTouch UltraMini meter (Johnson–Johnson, Brazil) in a drop of blood taken from a small cut on the tail tip. Lidocaine gel was applied to the tail tip to alleviate pain. Serum triglycerides and total cholesterol were determined in blood drawn from the tail, with colorimetric assay kits (Wiener, Rosario, Argentina).

2.4. Glucose tolerance test

Glucose (2 g/kg body weight) dissolved in saline was administered by intraperitoneal (*i.p.*) injection to overnight fasting animals. Glycemia (mg/dl) was measured prior to injection of glucose (time 0) and at 15 min, 30 min, 60 min and 90 min post-injection. For the calculation of the area under the curve (GraphPad Prism 5 software) baseline glycemia value was subtracted from subsequent readings. The area under the curve was expressed as Δ mg glucose/dl in 90 min.

2.5. Lipid peroxidation in the spinal cord

Lipid peroxidation in the spinal cord was estimated by the measurement of thiobarbituric acid reactive substances using the procedure described by Rodrigues et al. (2011) with modifications. The thoraco-lumbar portions of spinal cords of control and fructose-fed rats were homogenized (100 mg tissue/ml) in 20 mM potassium phosphate buffer containing 150 mM potassium chloride (pH 7.40). The homogenates were centrifuged at 4 °C (1000 g for 10 min) and aliquots of the supernatants (0.5 ml) were mixed with 4% butylated hydroxytoluene (15 µl) as antioxidant and 20% trichloroacetic acid (0.5 ml) to precipitate proteins and acidify the samples. The samples were centrifuged under the same conditions as before, and the supernatants plus 0.67% 2-thiobarbituric acid (1:1) incubated at 100 °C during 15 min. After cooling, the absorbance of the samples at 535 nm was determined in a spectrophotometer (Amersham Ultrospec 1100 Pro). The tissue levels of thiobarbituric acid reactive substances were calculated using 1,1,3,3-tetramethoxypropane as standard and expressed as nmol/mg protein. Total protein concentration in the homogenates was measured by the method of Bradford (1976).

2.6. Surgical procedures

Evaluation of the cardiovascular effects after either *i.t.* or intravenous (*i.v.*) injection of drugs was as previously described (García et al., 1998, 2006). On the day of the experiment, animals were anesthetized with sodium pentobarbital. The initial dose (40 mg/kg, *i.p.*) was supplemented with 10 mg/kg (*i.p.*) before the introduction of the *i.t.* catheter. Additional doses (2–4 mg/kg each time) were given during the experiment when necessary. The depth of anesthesia (surgical plane) was verified by the absence of the eyelid reflex. A polyethylene catheter was placed in the right femoral artery for blood pressure recording and, in some experiments, a femoral vein was cannulated for *i.v.* injection of drugs. For *i.t.* injection, a catheter (outside diameter: 0.65 mm) was inserted into the subarachnoid space at T_1–T_2 vertebrae level, and gently pushed downward 5.0 cm. The tip of the cannula was positioned at T_{12}–L_1 intervertebral space level. The position of the cannula was verified post-mortem by direct observation after opening the ventral aspect of the vertebrae. The body temperature was kept at 37–38 °C by a heating lamp.

2.7. Blood pressure recording and heart rate calculation

Blood pressure was measured at the right femoral artery via a Becton Dickinson transducer (model P23XL-1) and recorded on a Grass 7B polygraph. Baseline values were recorded for at least 30 min before starting the experiment. Mean blood pressure was calculated from the following formula: diastolic pressure +1/3 (systolic pressure–diastolic pressure). Heart rate was calculated from the blood pressure record. Changes in mean blood pressure and heart rate induced by *i.t.* injection of drugs refer to differences between values obtained just before the beginning of drug injection and values at a given time.

2.8. Experimental protocols

Intrathecal injections were administered with a Hamilton syringe, and consisted of 10 µl of one of the following drugs: anandamide (100 nmol), CGRP (0.125 nmol), noradrenaline (100 nmol); *N*-methyl-D-aspartate (NMDA; 30 nmol), sodium nitroprusside (500 nmol), muscimol (8.8 nmol), baclofen (100 nmol) or the corresponding vehicles. Additional saline (10 µl) was injected to wash the drug solutions from the catheter. The solutions (total volume 20 µl) were loaded into the syringe immediately before injection and were administered within 1 min. Blood pressure was recorded just before (time 0) and at the following time points after beginning *i.t.* injection of drugs: 1 min, 2 min, 4 min, 6 min, 8 min, 10 min, 15 min, 20 min, 25 min and 30 min. Doses of *i.t.* administered drugs were selected on the basis of previous studies (García et al., 1996, 1998, 2006; Celuch and García, 2002).

In an effort to reduce the number of animals used in the present study, blood pressure responses to *i.v.* injection of either

noradrenaline, acetylcholine or sodium nitroprusside were measured in animals previously used for *i.t.* injection of either vehicles or pressor compounds (*i.e.* noradrenaline and NMDA).

At the end of the experiments the animals were killed by cervical dislocation. The epididymal fat pads, one of the major visceral fat depots in rodents (Cinti, 2005), were excised from some animals and weighted.

2.9. Drugs

Anandamide was obtained from Cayman Chemical Co. (USA). Calcitonin gene-related peptide (CGRP), muscimol, sodium nitroprusside, *N*-methyl-D-aspartate (NMDA), noradrenaline bitartrate, acetylcholine bromide, butylated hydroxytoluene, 2-thiobarbituric acid and 1,1,3,3-tetramethoxypropane were obtained from Sigma Chemical Co. (USA). Baclofen was kindly donated by Ciba Geigy (Argentina).

Anandamide was supplied as ethanol solution and diluted with saline just before the injection. The final solution contained 14% ethanol (v/v). CGRP was dissolved in water. Lyophilized aliquots were stored at −20 °C and dissolved in saline before use. Other *i.t.* injected compounds were dissolved in saline, and aliquots were stored at −20 °C. Stock solution of butylated hydroxytoluene (4% in ethanol) was stored at 4 °C. 2-Thiobarbituric acid solution (0.67% in water) was prepared before use.

2.10. Statistics

All values represent mean ± S.E.M. Statistical differences between mean values at a given time were assessed by unpaired Student's *t*-test, or one-way ANOVA followed by Dunnett *post-hoc* test. *P* values smaller than 0.05 were regarded as significant.

3. Results

Rats maintained for 8 weeks with 10% fructose added to the drinking water showed a higher water intake than aged-matched control animals. Fructose feeding did not modify total body weight, but increased the epididymal fat weight (Table 1).

Blood concentration of glucose and serum levels of triglycerides were higher in fructose-fed than in control rats, whereas total serum cholesterol values were not altered by treatment. The area under curve value for the time-course of glycemia after an overload of glucose (2 g/kg, *i.p.*) was greater in fructose-fed than in control animals. Systolic blood pressure was moderately higher in conscious fructose-fed than in control animals, but no differences in either systolic or mean blood pressure were observed between groups during pentobarbital-induced anesthesia. Fructose treatment did not alter heart rate (Table 1).

3.1. Cardiovascular responses to drugs in fructose-fed and control animals

3.1.1. Intrathecal injections

Intrathecal injection of either the endocannabinoid anandamide (100 nmol) or the peptide CGRP (0.125 nmol) produced hypotensive effects in pentobarbital-anesthetized control rats (Fig. 1A and B), but did not modify the blood pressure in fructose-fed animals (Fig. 1C and D). On the other hand, fructose treatment did not alter the pressor response induced by *i.t.* injection of the adrenergic neurotransmitter noradrenaline (100 nmol) (Fig. 2A and C). Moreover, the pressor response to the excitatory amino acid NMDA (30 nmol) was slightly lower in fructose-fed rats than in control animals (Fig. 2B and D), but the difference between groups was not statistically significant (one-way ANOVA followed by Newman–Keuls *post-hoc* test).

The nitric oxide donor sodium nitroprusside (500 nmol, *i.t.*) induced a brief hypotension followed by a sustained increase in blood pressure in control rats (Fig. 3A); however, only the hypertensive effect was observed in fructose-fed animals (Fig. 3B). For a better comparison, Fig. 3C shows the early effects (from $t=0$ to $t=6$ min) of sodium nitroprusside in fructose treated vs. control rats.

As shown in Fig. 4, the $GABA_A$-receptor agonist muscimol (8.8 nmol, *i.t.*) and the $GABA_B$-receptor agonist baclofen (100 nmol, *i.t.*) decreased mean blood pressure 30–35 mmHg, both in control and in fructose-fed rats.

Table 2 shows changes in heart rate measured 2 min and 25 min after *i.t.* injection of drugs. Except for NMDA, which produced tachycardia in control as well as in treated rats, and for noradrenaline, which produced bradycardia in control animals during the first minutes post-injection, the rest of the drugs did not produce significant changes in heart rate.

3.1.2. Intravenous injections

Fructose treatment potentiated the pressor effect of *i.v.* injected noradrenaline, but did not modify the hypotensive responses to either sodium nitroprusside or acetylcholine (Fig. 5).

3.2. Lipid peroxidation in the spinal cord of fructose-fed and control animals

Tissue levels of thiobarbituric acid reactive substances, an index of lipid peroxidation and oxidative stress, were about 30% higher in spinal cords of fructose-fed animals than in the controls (Fig. 6).

4. Discussion

In agreement with previous studies that investigated the effect of high fructose diets in rodents (Renna et al., 2005; Takatori et al., 2006), we found that Sprague-Dawley rats receiving 10% fructose in the drinking water had lower glucose tolerance, higher daily water intake and higher glycemia and triglyceridemia values than those observed in control animals. Moreover, as reported previously (Renna et al., 2005; D'Angelo et al., 2005; Bi et al., 2008), no changes in serum total cholesterol levels were observed. Even if fructose feeding did not alter total body weight, the observation of heavier epididymal fat depots suggests that the treatment increased the body adipose tissue mass, probably due to hypertrophy of the adipocytes (Soria et al., 2001; Nagai et al., 2009). Although blood pressure in conscious fructose-fed rats was significantly higher than in control animals, no differences were found in pentobarbital-anesthetized animals. A possible explanation is that, as suggested by D'Angelo et al. (2005), the moderate hypertension registered in conscious fructose-fed rats by the tail-cuff plethysmography method may be related to an exaggerated stress response, or an impaired control of vascular resistance due to an increased basal sympathetic nerve activity.

The finding that *i.t.* injected anandamide, CGRP and sodium nitroprusside decreased the blood pressure in control rats but not in fructose-fed animals suggests that fructose treatment would be interfering with spinal mechanisms involved in the hypotensive responses to those compounds. We have previously shown that the hypotensive effects of *i.t.* injected anandamide and CGRP, as well as the short-lasting hypotensive response to *i.t.* administered sodium nitroprusside in anesthetized rats were prevented by either $GABA_A$- or $GABA_B$-receptor blockade in the spinal cord. Those findings lead us to suggest that the hypotensive responses observed would involve the release of GABA in the spinal cord

Table 1
Physiological parameters in control rats and in fructose-fed animals in animals treated for 8 weeks with 10% fructose in the drinking water.

	Control	*n*	10 % Fructose	*n*
Water intake (ml/day)[a]	48.2 ± 2.2	20	130.1 ± 4.1[d]	20
Body weight (g)	514.3 ± 12.9	20	542.7 ± 13.8	20
Epididymal fat (g)	7.2 ± 0.5	20	10.5 ± 0.6[c]	20
Epididymal fat (% body weight)	1.4 ± 0.1	20	1.9 ± 0.1[c]	20
Triglyceridemia (mg/dl)	85.63 ± 7.80	20	161.80 ± 14.57[d]	20
Total serum cholesterol (mg/dl)	66.98 ± 3.79	20	62.64 ± 3.59	20
Glycemia (mg/dl)	114.28 ± 3.28	20	142.22 ± 4.50[d]	20
Area under curve (Δ mg glucose/dl in 90 min)	7744 ± 630	7	13394 ± 1921[b]	7
Heart rate (beats/min) in conscious animals	352 ± 9	16	374 ± 10	16
Systolic blood pressure (mmHg) in conscious animals	136.1 ± 2.7	16	162.9 ± 3.9[d]	16
Systolic blood pressure (mmHg) in anesthetized animals	123.8 ± 3.9	20	125.8 ± 4.8	20
Mean blood pressure (mmHg) in anesthetized animals	97.5 ± 4.7	20	97.7 ± 4.9	20

Shown are mean values ± S.E.M.; *n*=number of animals.

[a] In this particular case, *n* is the number of data (see Section 2.1 in Materials and methods).
[b] $P < 0.05$.
[c] $P < 0.001$.
[d] $P < 0.0001$ vs. the corresponding control value (unpaired Student's *t*-test with Welch's correction).

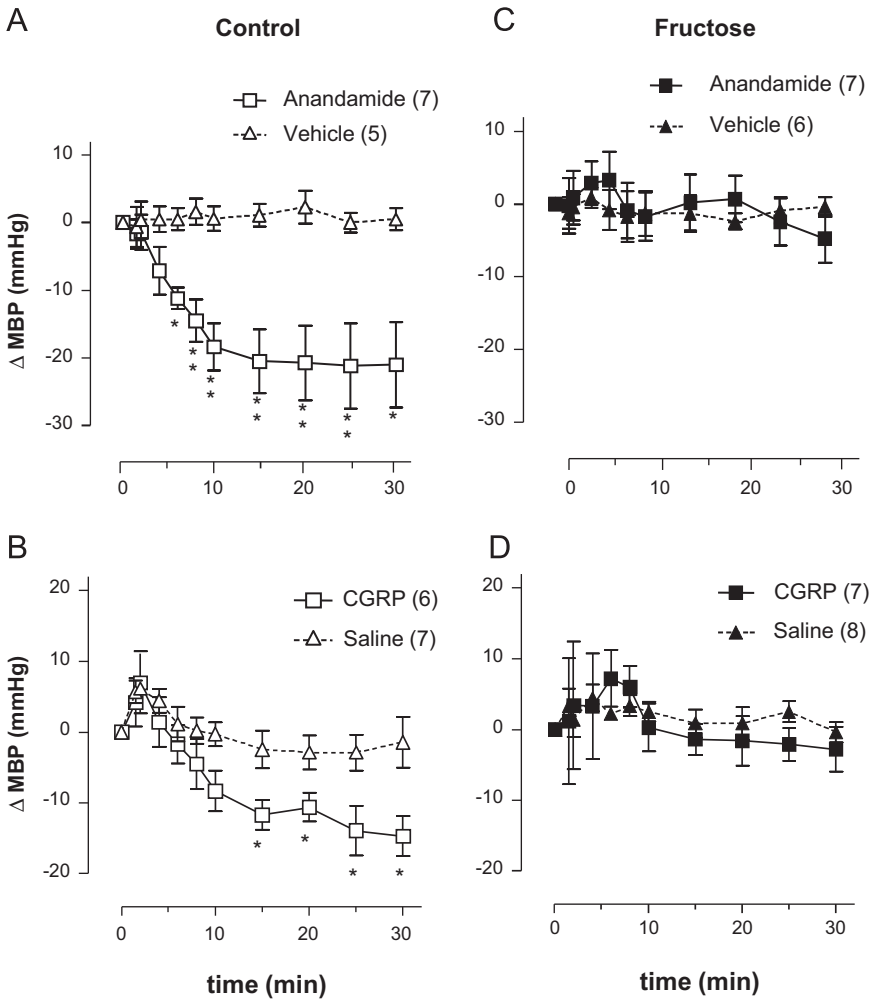

Fig. 1. Changes in mean blood pressure (ΔMBP; mmHg) induced by *i.t.* injection of anandamide (100 nmol) and CGRP (0.125 nmol) in control rats ((A), (B); open symbols) and in animals treated for 8 weeks with 10% fructose in the drinking water ((C), (D); filled symbols). Anandamide, CGRP, anandamide vehicle (14% ethanol in saline) and saline were injected at time 0. Shown are mean values ± S.E.M. Numbers in parenthesis indicate the number of animals per group. *$P < 0.05$; **$P < 0.005$ vs. the corresponding value in either the anandamide vehicle or the saline group (unpaired Student's *t*-test with Welch's correction).

(García et al., 1998, 2006). In this regard, it is well documented that GABA is a major inhibitory neurotransmitter for sympathetic preganglionic neurons in the spinal cord (Wu and Dun, 1992; Deuchars et al., 1997, 2005; Llewellyn-Smith, 2009; Wang et al., 2010). In the present study, treatment with fructose abolished the hypotensive responses to compounds that appear to produce their effects through spinal GABA release, but did not modify the hypotensive responses induced by either a $GABA_A$ or a $GABA_B$

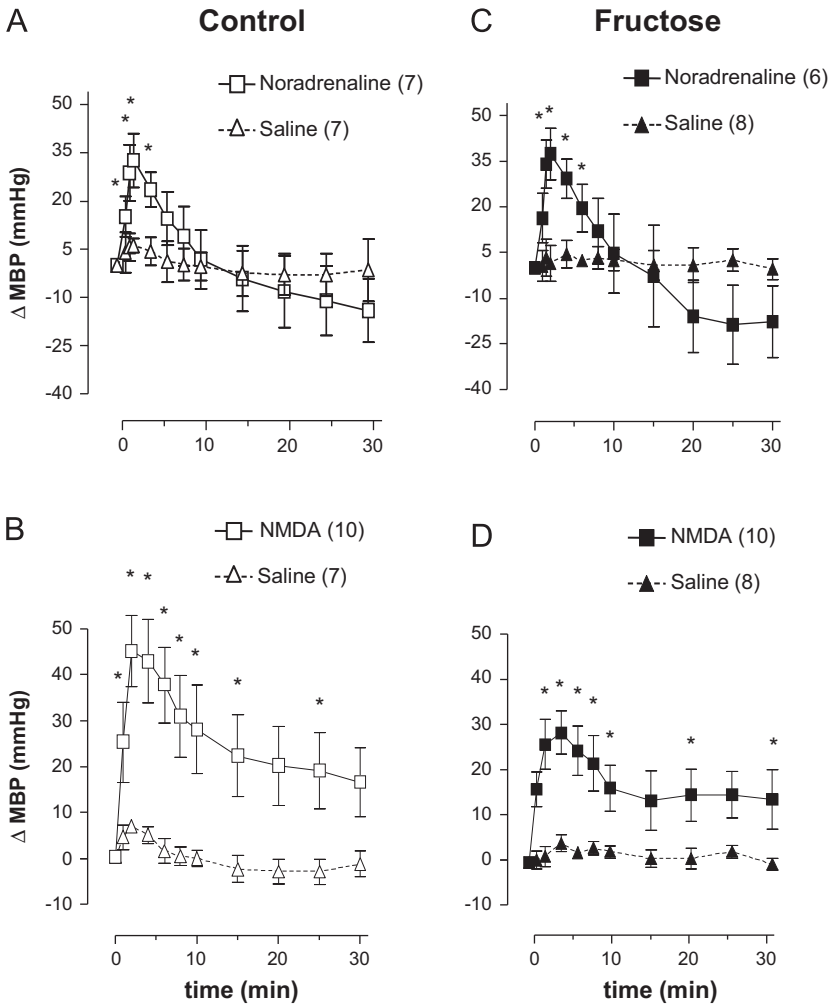

Fig. 2. Changes in mean blood pressure (ΔMBP; mmHg) induced by *i.t.* injection of noradrenaline (100 nmol) and NMDA (30 nmol) in control rats ((A), (B); open symbols) and in animals treated for 8 weeks with 10% fructose in the drinking water ((C), (D); filled symbols). Noradrenaline, NMDA and saline were injected at time 0. Shown are mean values ± S.E.M. Numbers in parenthesis indicate the number of animals per group. $^{*}P < 0.05$ vs. the corresponding saline value (unpaired Student's *t*-test with Welch's correction).

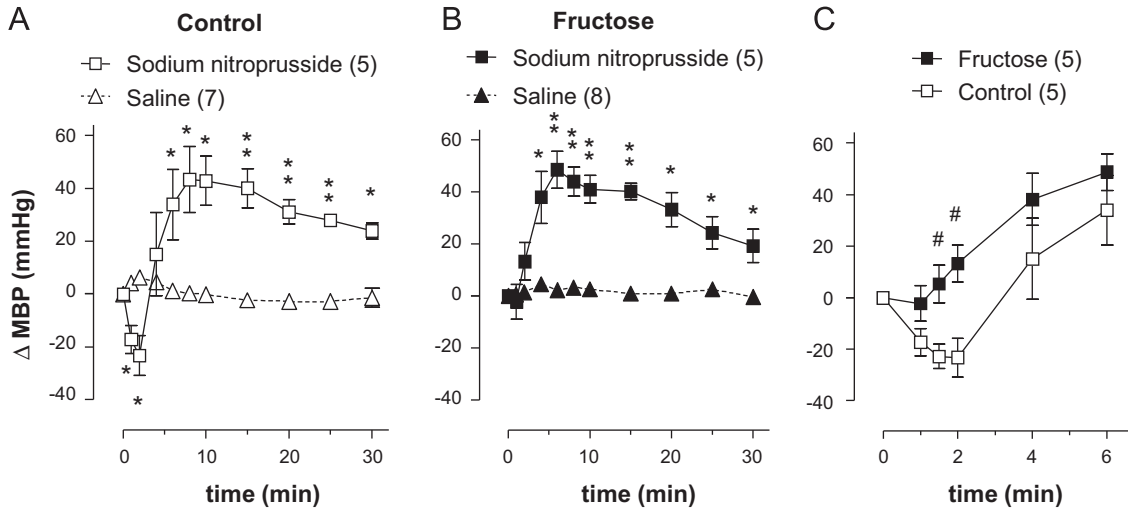

Fig. 3. Changes in mean blood pressure (ΔMBP; mmHg) induced by *i.t.* injection of sodium nitroprusside (500 nmol) in control rats ((A); open symbols) and in animals treated for 8 weeks with 10% fructose in the drinking water ((B); filled symbols). The responses to the drug during the first 6 min post-injection in both groups of animals are compared in (C). Sodium nitroprusside and saline were injected at time 0. Shown are mean values ± S.E.M. Numbers in parenthesis indicate the number of animals per group. $^{*}P < 0.05$; $^{**}P < 0.005$ vs. the corresponding saline value; $^{\#}P < 0.05$ vs. the corresponding control value (unpaired Student's *t*-test with Welch's correction).

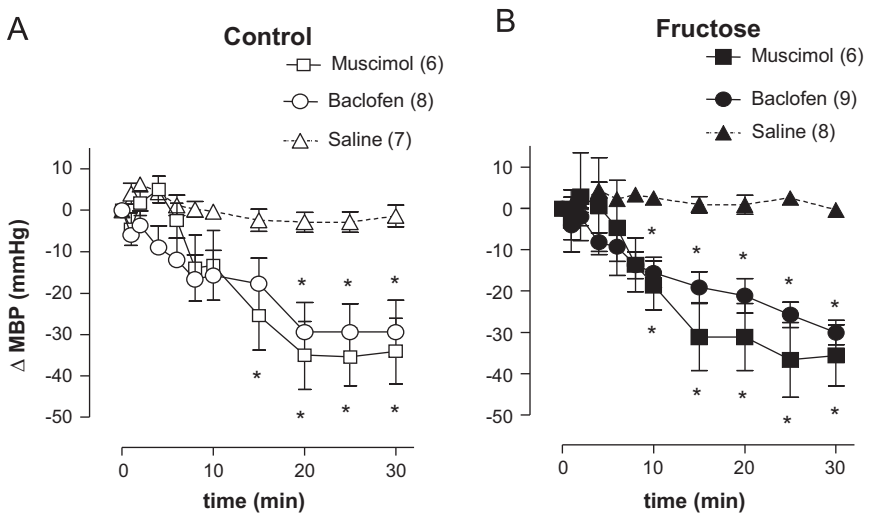

Fig. 4. Changes in mean blood pressure (ΔMBP; mmHg) induced by *i.t.* injection of muscimol (8.8 nmol) and baclofen (100 nmol) in control rats ((A); open symbols) and in animals treated for 8 weeks with 10% fructose in the drinking water ((C); filled symbols). Muscimol, baclofen and saline were injected at time 0. Shown are mean values ± S.E.M. Numbers in parenthesis indicate the number of animals per group. $^{*}P < 0.05$ vs. the corresponding saline value (one way ANOVA followed by Dunnett *post-hoc* test).

Table 2
Effects of *i.t.* administered compounds on the heart rate of control rats and animals receiving 10% fructose in the drinking water during 8 weeks.

Compound	Control				10% Fructose			
	Baseline HR[a] (beats/min)	ΔHR 2 min[b] (beats/min)	ΔHR 25 min[c] (beats/min)	*n*	Baseline HR[a] (beats/min)	ΔHR 2 min[b] (beats/min)	ΔHR 25 min[c] (beats/min)	*n*
Anandamide vehicle	396 ± 12	3 ± 3	3 ± 3	5	360 ± 14	−16 ± 6	−13 ± 9	6
Anandamide	399 ± 23	−16 ± 6	11 ± 9	7	390 ± 16	3 ± 5	−7 ± 6	7
saline	416 ± 12	−7 ± 6	−1 ± 7	7	347 ± 40	−1 ± 5	1 ± 6	8
CGRP	370 ± 18	−2 ± 7	8 ± 17	6	370 ± 18	−6 ± 8	10 ± 14	7
Muscimol	348 ± 13	2 ± 7	−40 ± 18	6	370 ± 16	−23 ± 13	−35 ± 17	6
Baclofen	376 ± 8	−6 ± 4	−30 ± 17	8	394 ± 13	−6 ± 7	−36 ± 14	9
Noradrenaline	360 ± 16	−53 ± 14[d]	−24 ± 28	7	362 ± 12	−30 ± 20	10 ± 7	6
NMDA	387 ± 20	20 ± 13	43 ± 11[d]	10	399 ± 15	50 ± 16[d]	58 ± 21[d]	10
Sodium nitroprusside	410 ± 9	18 ± 7	−18 ± 19	5	346 ± 20	18 ± 9	40 ± 17	5

[a] Heart rate (HR) just prior to *i.t.* injection of the following drugs or the corresponding vehicles: 100 nmol anadamide ; 0.125 nmol CGRP; 8.8 nmol muscimol, 100 nmol baclofen; 100 nmol noradrenaline, 30 nmol NMDA; 500 nmol sodium nitroprusside. Changes in HR (ΔHR) were measured 2 min.
[b] 25 min.
[c] Post-injection. Shown are mean values ± S.E.M.; *n* = number of observations per group.
[d] $P < 0.05$ vs. saline (one way ANOVA followed by Dunnett *post-hoc* test).

receptor agonist (Fig. 4). It is thus proposed that fructose feeding may alter some spinal inhibitory mechanism, at a stage prior to the activation of GABA receptors involved in the modulation of sympathetic preganglionic neuron activity.

Diabetic neuropathies are associated to an increase in oxidative/nitrosative stress in the spinal cord as well as in dorsal ganglia and nerves (Vareniuk et al., 2007; Ismael et al., 2008). Furthermore, it has been reported that reactive oxygen species contribute to neuropathic pain by reducing spinal GABA release (Yowtak et al., 2011). Therefore, since sugar rich diets produce oxidative stress (Renna et al., 2005; Ismael et al., 2008; Oudot et al., 2009), the possibility exists that the lack of hypotensive effect of anandamide, CGRP and nitroprusside in fructose-fed rats could be related to some impairment in spinal GABAergic neurotransmission, due to the presence of oxidative species. In fact, this study shows that fructose feeding enhanced the production of thiobarbituric acid reactive substances in the spinal cord. In this regard, it will be of interest to analyze in the future whether fructose feeding modifies GABA tissue content or its release in animals treated for 8 weeks with 10% fructose in the drinking water and whether the blood pressure responses to *i.t.* injected compounds in control and fructose treated rats are modified by acutely or chronically administered antioxidants and free radical scavengers and donors.

In contrast to the findings with anandamide, CGRP and the early effect of sodium nitroprusside, fructose treatment did not cause significant changes in the pressor responses to *i.t.* administered substances that exert excitatory effects at sympathetic preganglionic neurons, such as noradrenaline, NMDA and glutamate. The latter excitatory amino acid is involved, according to a previous study (García et al., 1998), in the hypertensive response to *i.t.* injected sodium nitroprusside in anesthetized rats. Given that the pressor responses to *i.t.* injected compounds are a consequence of the release of catecholamines from perivascular sympathetic nerve endings and adrenal medulla, and since it was found that fructose treatment enhanced the vasoconstrictor effects of noradrenaline (Fig. 5A), we suggest that the pressor responses to *i.t.* injected noradrenaline and excitatory amino acids could be, in fact, attenuated by the treatment, and that the higher vascular reactivity to catecholamines counterbalanced

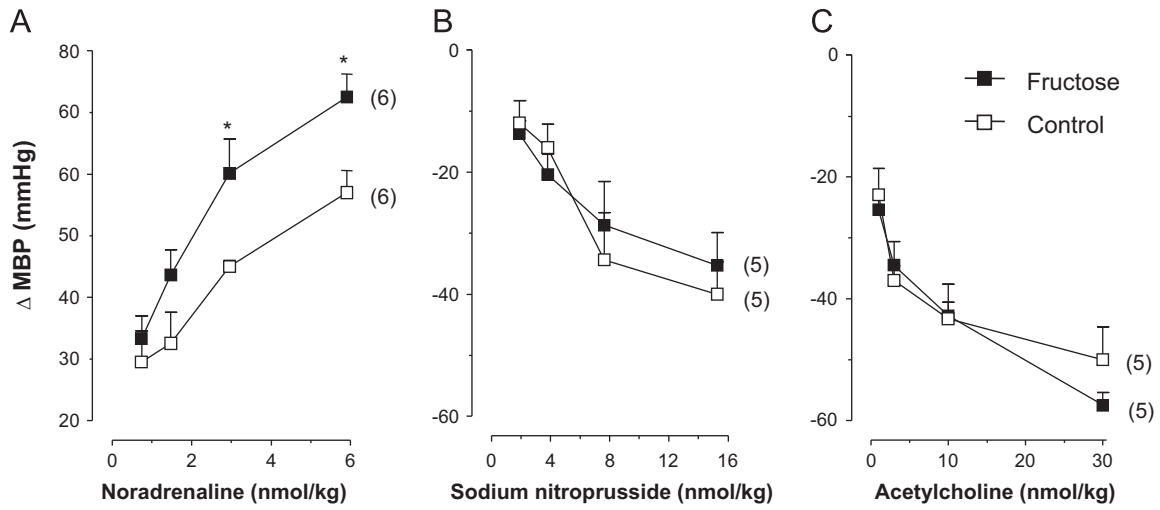

Fig. 5. Changes in mean blood pressure (ΔMBP; mmHg) induced by *i.v.* injection of increasing doses of (A) noradrenaline, (B) sodium nitroprusside and (C) acethylcholine in control rats (open symbols) and in animals treated for 8 weeks with 10% fructose in the drinking water (filled symbols). Shown are mean values ± S.E.M. Numbers in parenthesis indicate the number of animals per group. *$P < 0.05$ vs. the corresponding control value. (unpaired Student's *t*-test with Welch's correction).

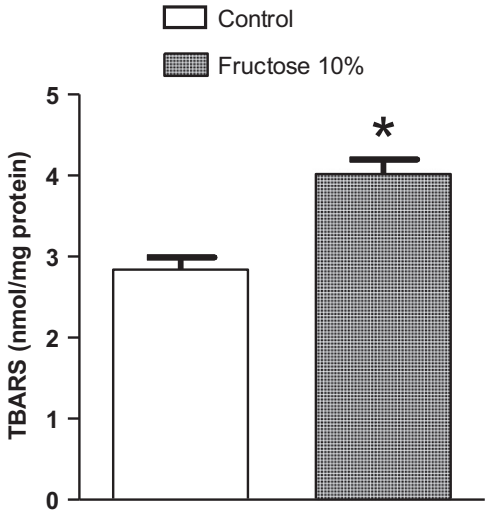

Fig. 6. Tissue levels (nmol/mg protein) of thiobarbituric acid reactive substances (TBARS) in the spinal cord of control rats and in animals treated for 8 weeks with 10% fructose in the drinking water. Shown are mean values ± S.E.M. of five animals per group. *$P < 0.02$ vs. the control value (unpaired Student's *t*-test).

this decrease. As proposed above, it could be interesting to investigate whether this is related to oxidative stress.

Taken together, the results obtained herein with *i.t.* injected compounds in pentobarbital-anesthetized rats suggest that fructose feeding produces alterations in the spinal mechanisms of regulation of preganglionic sympathetic nerve activity that might contribute to sympathetic dysfunction in this model of metabolic syndrome.

It is a well-established fact that anesthetics affect cardiovascular functions (Fluckiger et al., 1985, Shimokawa et al., 1998; Kurz et al., 2009). Therefore, at the present, it cannot be assessed whether the results observed in pentobarbital-anesthetized rats could be also obtained in conscious animals or with a different anesthetic.

The observation that the pressor responses to *i.v.* administered noradrenaline were greater in fructose-fed rats is in agreement with previous studies, which suggest that the higher vascular reactivity is related to either an increase in circulating insulin (Takatori et al., 2003, 2006) or an increase in the production of vasoconstrictor prostanoids (Oudot et al., 2009). On the other hand, fructose treatment did not appear to alter either the endothelium-dependent or endothelium-independent vasorelaxation as the hypotensive responses induced by acetylcholine and sodium nitroprusside, respectively, remained unaltered. The result obtained with acetylcholine is in contradiction with a number of studies that reported impaired endothelial-dependent vasorelaxation associated with metabolic syndrome and type 2 diabetes, both in humans (Hamburg et al., 2008; Bai et al., 2012) and in experimental animal models, including the fructose-fed rat (Bartuś et al., 2005; Romanko et al., 2009; Zhong et al., 2012). Nevertheless, our observation is in agreement with other reports showing that endothelium-dependent vasorelaxation induced by acetylcholine remained unchanged either in fructose-fed (Takatori et al., 2003, 2006, Patel et al., 2009) or in Zucker diabetic fatty rats (Pamarthi et al., 2002). The reasons for the discrepancies between studies are not clear, but might be related to several factors such as rat strain and breeding, daily ingestion of fructose and other nutrients, duration of the treatment and other experimental conditions such as the use of anesthetics and whether the assay was performed on either the whole animal or isolated arteries. Interestingly, Oudot et al. (2009) suggested that the unaltered vasorelaxing effect of acetylcholine in fructose treated rats could be the result of an enhanced production of endothelial vasodilator prostanoids that masked the decreased production of other endothelial vasodilators, such as nitric oxide.

The observation that most of the *i.t.* injected substances produced no consistent changes in heart rate suggests that there was no significant diffusion of compounds from the injection site at T_{12}–L_1 up to the T_1–T_3 level, where the preganglionic sympathetic neurons that innervate the heart are localized (Sundaram et al., 1989). In fact, very low radioactivity was found in the T_1–T_3 segment after *i.t.* injection of either tritiated anandamide (García et al., 2009) or tritiated glutamate (Celuch, S.M.; unpublished observation) at the thoraco-lumbar level of the spinal cord. Even so, the increase of heart rate obtained with NMDA suggests that this potent excitatory amino acid reach the T_1–T_3 level at concentrations high enough as to stimulate preganglionic sympathetic neurons involved in cardiac innervation. The slight but significant bradycardia produced by 100 nmol noradrenaline in control rats might be a reflex response to baroreceptor activation, although it is to be noted that this compensatory mechanism was probably attenuated by the anesthetic (Fluckiger et al., 1985; Barringer and Buñag, 1990; Shimokawa et al., 1998).

In conclusion, the present study suggests that in pentobarbital-anesthetized rats fructose feeding could alter spinal mechanisms of

regulation of preganglionic sympathetic nerve activity. It is proposed that the spinal cord could be involved in the sympathetic dysfunction associated with the metabolic syndrome.

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References

Bai, Q., Lai, X., Zhang, A.H., Lu, X.H., Tian, S.L., Fan, M.H., Wang, Y., Wang, T., 2012. Metabolic syndrome and its components associated with endothelial dysfunction in chronic kidney disease patients. Vasc. Health Risk Manag. 8, 15–21.

Barringer, D.L., Buñag, R.D., 1990. Differential anesthetic depression of chronotropic baroreflexes in rats. J. Cardiovasc. Pharmacol. 15, 10–15.

Bartuś, M., Lomnicka, M., Lorkowska, B., Franczyk, M., Kostogrys, R.B., Pisulewski, P.M., Chłopicki, S., 2005. Hypertriglyceridemia but not hypercholesterolemia induces endothelial dysfunction in the rat. Pharmacol. Rep. 57, 127–137.

Basciano, H., Federico, L., Adeli, K., 2005. Fructose, insulin resistance, and metabolic dyslipidemia. Nutr. Metab. 2, 5.

Bi, X.P., Tan, H.W., Xing, S.S., Wang, Z.H., Tang, M.X., Zhang, Y., Zhang, W., 2008. Overexpression of TRB3 gene in adipose tissue of rats with high fructose-induced metabolic syndrome. Endocr. J. 55, 747–752.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt. Biochem. 72, 248–254.

Celuch, S.M., García, M.C., 2002. Activation of spinal metabotropic glutamate receptors elicits cardiovascular responses in pentobarbital anesthetized rats. Naunyn-Schmiedeberg's Arch. Pharmacol. Toxicol. 366, 343–349.

Cinti, S., 2005. The adipose organ. Prostaglandins, Leukot. Essent. Fatty Acids 73, 9–15.

Coote, J.H., 1988. The organisation of cardiovascular neurons in the spinal cord. Rev. Physiol. Biochem. Pharmacol. 110, 147–285.

D'Angelo, G., Elmarakby, A.A., Pollock, D.M., Stepp, D.W., 2005. Fructose feeding increases insulin resistance but not blood pressure in Sprague-Dawley rats. Hypertension 46, 806–811.

Deedwania, P., 2011. Hypertension, dyslipidemia, and insulin resistance in patients with diabetes mellitus or the cardiometabolic syndrome: benefits of vasodilating β-blockers. J. Clin. Hypertens. 13, 52–59.

Després, J.P., Lemieux, I., Bergeron, J., Pibarot, P., Mathieu, P., Larose, E., Rodés-Cabau, J., Bertrand, O.F., Poirier, P., 2008. Abdominal obesity and the metabolic syndrome: contribution to global cardiometabolic risk. Arterioscler. Thromb. Vasc. Biol. 28, 1039–1049.

Deuchars, S.A., Milligan, C.J., Stornetta, R.L., Deuchars, J., 2005. GABAergic neurons in the central region of the spinal cord: a novel substrate for sympathetic inhibition. J. Neurosci. 25, 1063–1070.

Deuchars, S.A., Spyer, K.M., Gilbey, M.P., 1997. Stimulation within the rostral ventrolateral medulla can evoke monosynaptic GABAergic IPSPs in sympathetic preganglionic neurons in vitro. J. Neurophysiol. 77, 229–235.

Farah, V., Elased, K.M., Chen, Y., Key, M.P., Cunha, T.S., Irigoyen, M.C., Morris, M., 2006. Nocturnal hypertension in mice consuming a high fructose diet. Auton. Neurosci. 130, 41–50.

Fluckiger, J.P., Sonnay, M., Boillat, N., Atkinson, J., 1985. Attenuation of the baroceptor reflex by general anesthetic agents in the normotensive rat. Eur. J. Pharmacol. 109, 105–109.

García, M.C., Adler-Graschinsky, E., Celuch, S.M., 2006. Role of CGRP and GABA in the hypotensive effect of intrathecally administered anandamide to anesthetized rats. Eur. J. Pharmacol. 532, 88–98.

García, M.C., Adler-Graschinsky, E., Celuch, S.M., 2009. Enhancement of the hypotensive effects of intrathecally injected endocannabinoids by the entourage compound palmitoylethanolamide. Eur. J. Pharmacol. 610, 75–80.

García, M.C., Celuch, S.M., Adler-Graschinsky, E., 1998. Involvement of GABA and glutamate receptors in the blood pressure responses to intrathecally injected sodium nitroprusside in anesthetized rats. Eur. J. Pharmacol. 349, 245–252.

García, M.C., Enero, M.A., Celuch, S.M., 1996. Hypotensive and hypertensive effects of catecholamines intrathecally injected in anesthetized rats. J. Auton. Nerv. Syst. 59, 17–26.

Grassi, G., Arenare, F., Quarti-Trevano, F., Seravalle, G., Mancia, G., 2009. Heart rate, sympathetic cardiovascular influences, and the metabolic syndrome. Prog. Cardiovasc. Dis. 52, 31–37.

Hamburg, N.M., Larson, M.G., Vita, J.A., Vasan, R.S., Keyes, M.J., Widlansky, M.E., Fox, C.S., Mitchell, G.F., Levy, D., Meigs, J.B., Benjamin, E.J., 2008. Metabolic syndrome, insulin resistance, and brachial artery vasodilator function in Framingham Offspring participants without clinical evidence of cardiovascular disease. Am. J. Cardiol. 101, 82–88.

Ismael, M.A., Talbot, S., Carbonneau, C.L., Beauséjour, C.M., Couture, R., 2008. Blockade of sensory abnormalities and kinin B(1) receptor expression by *N*-acetyl-L-cysteine and ramipril in a rat model of insulin resistance. Eur. J. Pharmacol. 589, 66–72.

Kishi, T., Hirooka, Y., Ogawa, K., Konno, S., Sunagawa, K., 2011. Calorie restriction inhibits sympathetic nerve activity via anti-oxidant effect in the rostral ventrolateral medulla of obesity-induced hypertensive rats. Clin. Exp. Hypertens. 33, 240–245.

Kurz, C., Baranowska, U., Lupiński, S., Göthert, M., Malinowska, B., Schlicker, E., 2009. Urethane, but not pentobarbitone, attenuates presynaptic receptor function in rats: a contribution to the choice of anaesthetic. Br. J. Pharmacol. 157, 1474–1482.

Llewellyn-Smith, I.J., 2009. Anatomy of synaptic circuits controlling the activity of sympathetic preganglionic neurons. J. Chem. Neuroanat. 38, 231–239.

Mancia, G., Bousquet, P., Elghozi, J.L., Esler, M., Grassi, G., Julius, S., Reid, J., Van Zwieten, P.A., 2007. The sympathetic nervous system and the metabolic syndrome. J. Hypertens. 25, 909–920.

Mayer, M.A., Höcht, C., Gironacci, M., Opezzo, J.A., Taira, C.A., Fernández, B.E., Puyó, A.M., 2008. Hypothalamic angiotensinergic-noradrenergic systems interaction in fructose induced hypertension. Regul. Pept. 146, 38–45.

Mayer, M.A., Höcht, C, Opezzo, JA, Peredo, HA, Navacchia, D, Taira, CA., Fernández, B.E., Puyó, A.M., 2006. Role of hypothalamic alpha-adrenoceptor activity in fructose-induced hypertension. Clin. Exp. Pharmacol. Physiol. 33, 904–909.

Nagae, A., Fujita, M., Kawarazaki, H., Matsui, H., Ando, K., Fujita, T., 2009. Sympathoexcitation by oxidative stress in the brain mediates arterial pressure elevation in obesity-induced hypertension. Circulation 119, 978–986.

Nagai, Y., Ichihara, A., Nakano, D., Kimura, S., Pelisch, N., Fujisawa, Y., Hitomi, H., Hosomi, N., Kiyomoto, H., Kohno, M., Ito, H., Nishiyama, A.I., 2009. Possible contribution of the non-proteolytic activation of prorenin to the development of insulin resistance in fructose-fed rats. Exp. Physiol. 94, 1016–1023.

Oudot, A., Behr-Roussel, D., Compagnie, S., Caisey, S., Le Coz, O., Gorny, D., Alexandre, L., Giuliano, F., 2009. Endothelial dysfunction in insulin-resistant rats is associated with oxidative stress and COX pathway dysregulation. Physiol. Res. 58, 499–509.

Pamarthi, M.F., Rudd, M.A., Bukoski, R.D., 2002. Normal perivascular sensory dilator nerve function in arteries of Zucker diabetic fatty rats. Am. J. Hypertens. 15, 310–315.

Patel, J., Iyer, A., Brown, L., 2009. Evaluation of the chronic complications of diabetes in a high fructose diet in rats. Indian J. Biochem. Biophys. 46, 66–72.

Reaven, G., 2002. Metabolic syndrome: pathophysiology and implications for management of cardiovascular disease. Circulation 106, 286–288.

Renna, N., Risler, N., Cruzado, M., Gonzalez, S., Lama, C., Miatello, R.M., 2005. Effect of nebivolol on cardiovascular changes associated with a rat model of insulin-resistance. Cell. Mol. Biol. 51, 531–537.

Rodrigues, B., Figueroa, D.M., Fang, J., Rosa, K.T., Llesuy, S., De Angelis, K., Irigoyen, M.C., 2011. Short-term diabetes attenuates left ventricular dysfunction and mortality rates after myocardial infarction in rodents. Clinics (Sao Paulo) 66, 1437–1442.

Romanko, O.P., Ali, M.I., Mintz, J.D., Stepp, D.W., 2009. Insulin resistance impairs endothelial function but not adrenergic reactivity or vascular structure in fructose-fed rats. Microcirculation 16, 414–423.

Shimokawa, A., Kunitake, T., Takasaki, M., Kannan, H., 1998. Differential effects of anesthetics on sympathetic nerve activity and arterial baroreceptor reflex in chronically instrumented rats. J. Automon. Nerv. Syst. 72, 46–54.

Soria, A., D'Alessandro, M.E., Lombardo, Y.B., 2001. Duration of feeding on a sucrose-rich diet determines metabolic and morphological changes in rat adipocytes. J. Appl. Physiol. 91, 2109–2116.

Sundaram, K., Murugaian, J., Krieger, A., Sapru, H., 1989. Microinjections of cholinergic agonists into the intermediolateral cell column of the spinal cord at T_1–T_3 increase heart rate and contractility. Brain Res. 503, 22–31.

Takatori, S., Mizote, M., Zamami, Y., Kurosaki, Y., Kawasaki, H., 2003. Effects of insulin on vascular responses to spinal cord stimulation and vasoactive agents in pithed rats. Br. J. Pharmacol. 140, 1137–1145.

Takatori, S., Zamami, Y., Mio, M., Kurosaki, Y., Kawasaki, H., 2006. Chronic hyperinsulinemia enhances adrenergic vasoconstriction and decreases calcitonin gene-related peptide-containing nerve-mediated vasodilation in pithed rats. Hypertens. Res. 29, 361–368.

Tang, X., Neckel, N.D., Schramm, L.P., 2004. Spinal interneurons infected by renal injection of pseudorabies virus in the rat. Brain Res. 1004, 1–7.

Vareniuk, I., Pavlov, I.A., Drel, V.R., Lyzogubov, V.V., Ilnytska, O., Bell, S.R., Tibrewala, J., Groves, J.T., Obrosova, I.G., 2007. Nitrosative stress and peripheral diabetic neuropathy in leptin-deficient (ob/ob) mice. Exp. Neurol. 205, 425–436.

Wang, L., Bruce, G., Spary, E., Deuchars, J., Deuchars, S.A., 2010. GABA(B) mediated regulation of sympathetic preganglionic neurons: pre- and postsynaptic sites of action. Front. Neurol. 1, 142.

Wu, S.Y., Dun, N.J., 1992. Presynaptic GABAB receptor activation attenuates synaptic transmission to rat sympathetic preganglionic neurons in vitro. Brain Res. 572, 94–102.

Yowtak, J., Lee, K.Y., Kim, H.Y., Wang, J., Kim, H.K., Chung, K., Chung, J.M., 2011. Reactive oxygen species contribute to neuropathic pain by reducing spinal GABA release. Pain 152, 844–852.

Zhong, M.F., Shen, W.L., Tabuchi, M., Nakamura, K., Chen, Y.C., Qiao, C.Z., He, J., Yang, J., Zhang, C., Kamenov, Z., Higashino, H., Chen, H., 2012. Differential changes of aorta and carotid vasodilation in type 2 diabetic GK and OLETF rats: paradoxical roles of hyperglycemia and insulin. Exp. Diabetes Res. 2012, 429020.