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Soluble guanylate cyclase chronic stimulation effects on cardiovascular reactivity in cafeteria diet-induced rat model of metabolic syndrome

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Abstract

Metabolic syndrome is linked to an increased risk of cardiovascular complications by a mechanism involving mainly decreased nitric oxide (NO) bioavailability and impaired NO-soluble guanylate cyclase (sGC)- cyclic guanosine monophosphate (cGMP) signalling (NO-sGC-cGMP). To further develop this scientific point, this study aimed to investigate the effects of long-term treatment with BAY 41-2272 (a sGC stimulator) on cardiovascular reactivity of spontaneously hypertensive rats (SHR) as a model of metabolic syndrome. SHR were randomly divided into 3 groups: Control group, cafeteria diet (CD)-fed group and CD-fed group treated daily with BAY 41-2272 (5mg/Kg) by gastric gavage for 12 weeks. *In vivo* measurements of body weight, abdominal circumference, blood pressure and glucose tolerance test were performed. At the end of the feeding period, *ex vivo* cumulative concentration-response curves were performed on isolated perfused heart (isoproterenol (0.1nM - 1µM)) and thoracic aorta (phenylephrine (1nM – 10 µM), acetylcholine (1nM – 10 µM), and sodium nitroprusside (SNP) (0.1nM – 0.1µM)). We showed that chronic CD feeding induced abdominal obesity, hypertriglyceridemia, glucose intolerance and exacerbated arterial hypertension in SHR. Compared to control group, CD-fed group showed a decrease in β-adrenoceptor-induced cardiac inotropy, in coronary perfusion pressure and in aortic contraction to phenylephrine. While relaxing effects of acetylcholine and SNP were unchanged. BAY 41-2272 long-term treatment prevented markedly arterial hypertension development and glucose intolerance, enhanced the α_1-adrenoceptor-induced vasoconstriction, and restored cardiac inotropy and coronary vasodilation. These findings suggest that BAY 41-2272 may be a potential novel drug for preventing metabolic and cardiovascular complications of metabolic syndrome.

Key words: metabolic syndrome, sGC stimulation, SHR, Cafeteria diet, cardiovascular reactivity

1. Introduction

Metabolic syndrome consists of a combination of cardiovascular risk factors such as obesity, insulin resistance, arterial hypertension, dyslipidaemia and an impaired glucose tolerance (O'Neill and O'Driscoll, 2015). Individuals with metabolic syndrome are at significant risk of developing cardiovascular disease and type II diabetes mellitus (Galassi et al., 2006; Aschner, 2010). Endothelial dysfunction characterized by an impaired endothelium-dependent vasodilation, is a predictive hallmark of later cardiovascular complications such stroke and heart attack (Shayo et al., 2019). Currently, an association is clearly established between endothelial dysfunction and metabolic syndrome (Abd El Aziz et al., 2018). The endothelium generates several vasoactive compounds and signals which act locally to adjust blood flow including nitric oxide (NO) (Fernandes et al., 2017; Khaddaj Mallat et al., 2017). Once released, NO diffuses into the smooth muscle cell, activates soluble guanylate cyclase (sGC) thereby generating cyclic guanosine monophosphate (cGMP) synthesis. cGMP induces vasorelaxation via lowering intracellular calcium levels (Kim et al., 2019). It has been reported that endothelial dysfunction associated with the metabolic syndrome is mainly due to the reduced NO bioavailability and therefore to an impairment in NO-sGC-cGMP signalling pathway (Matthews et al., 2018; Breitenstein et al., 2017). However, recent studies have shown that chronic treatment with sildenafil citrate, a phosphodiesterase type 5 (PDE5) inhibitor, improves energy balance contributing to weight loss in high fat-fed mice (Ayala et al., 2007), reduces hyperinsulinemia and up-regulates endothelial nitric oxide synthase (eNOS) expression in a rat model of insulin resistance (Oudot et al., 2010). Those findings suggest that the NO-cGMP pathway modulation may constitute a key link between its metabolic and vascular protective effects. Nonetheless, basal endogenous cGMP production is a prerequisite for the PDE5 inhibitors action (Tobin et al., 2018). Currently, different classes of drugs have been developed, which increase cGMP production independently of NO

availability, by targeting the NO receptor sGC (Breitenstein et al., 2017). Thus, sGC stimulation may have a crucial advantage over PDE5 inhibition due to its NO-independent mechanism of action (Chamorro et al., 2018), especially since sGC activity has been described as reduced in spontaneously hypertensive rats (SHR) (Priviero et al., 2009). BAY 41-2272 is a sGC stimulator that has been shown to induce antihypertensive action, to attenuate remodelling in models of systemic arterial hypertension and to reduce pulmonary vascular resistance (Boerrigter and Burnett, 2007). Furthermore, potential antiobesity and insulin sensitizing effects of cGMP signalling have been postulated (Mitschke et al., 2013). However, the cardiovascular effects of BAY 41-2272 long-term treatment during metabolic syndrome are not well known yet.

Several experimental models are now available for studying the pathogenesis and prevention of metabolic syndrome (Miesel et al., 2010). The SHR are one of the most commonly animal models studied (Oron-Herman et al., 2008). This strain is not only genetically hypertensive but is also insulin resistant (Potenza et al., 2005), thus representing a relevant study model of the metabolic syndrome in humans.

Thus, the present study was designed to investigate the effects of long-term sGC stimulation with BAY 41-2272 on metabolic parameters and cardiovascular reactivity in a SHR experimental model of metabolic syndrome.

2. Materials and methods

2.1.Animals and experimental protocol

All the experiments were performed in accordance with institutional guidelines from the ethical committee of Pays de la Loire, France (Ministry authorisation, APAFIS N° 6445). Nine-week old, male SHR obtained from Janvier Labs (Le Genest St Isle, France), were used for this study. All rats were housed under a 12-hour light/dark cycle, at a controlled

temperature (22°C) and humidity (50%) and were allowed free access to standard chow (KLIBA NAFAG®, Kaiseraugst, Germany) and drinking water. An acclimatisation period of 1 week was allowed before any experiment was initiated. Rats were randomly divided into 3 groups that received respectively for 12 weeks: standard chow, Cafeteria diet (CD) and CD with BAY 41-2272 administered orally (5mg/kg/day). CD included different commercial variety of chocolate, cookie and cereal bars. The resulting CD provided an average of 68.3% energy from carbohydrates, 5.83% from protein and 18.2% from total fat. The foods provided were changed daily to stimulate hyperphagia.

2.2.Physiological parameters

Body weight and abdominal circumference of all groups were monitored weekly during the feeding period.

Measurements of systolic (SBP) and diastolic (DBP) arterial blood pressures were assessed by non-invasive tail cuff plethysmography method in awake rats (CODA, Kent Scientific Co., Torrington, CT, USA). In order to limit stress-related variations in blood pressure, all measurements were performed by the same person and in a quiet room and rats were subjected to an adaptation period of one week before data collection.

Before starting measurements, rats were placed in a restraining box, preheated at 37° C in order to dilate the tail arteries. Ten consecutive pressure measurements were recorded for each rat and averaged to obtain a representative value of SBP and DBP (mmHg).

2.3.Glucose tolerance test and biochemical measurements

At the 12^{th} week, rats were fasted overnight. An intraperitoneal glucose tolerance test was carried out by means of glucose solution injection (1g/kg body weight, intraperitoneally). Blood sampling (one drop) was performed from tail vein (under ointment lidocaine

application) before and at 15, 30, 45, 60 and 90 min after glucose injection. The concentration of blood glucose was determined with a blood glucose meter (Glucometer, Pura ®).

At the end of the experimental protocol, animals were anesthetized with pentobarbital (54mg/kg i.p). Anaesthesia of the rat was checked by the paw withdrawal reflex. Blood samples were obtained via cardiac puncture and centrifuged at 5000g for 10 min at 4°C. Plasma was extracted and stored at -80°C. Total cholesterol and triglycerides plasma concentrations were assayed using an automatic biochemical analyser in the Veterinary University Hospital Centre of ONIRIS, Nantes, France. Insulin plasma concentrations were determined using a rat insulin ELISA kit (Thermo Fisher Scientific, France),

2.4.*Ex vivo* cardiac function

Immediately after blood sampling, rats were killed by exsanguination of abdominal aorta. The hearts were thoroughly excised and immersed in a cold *Krebs-Henseleit* solution (in mM): NaCl, 118.3; KCL, 4.7; $MgSO_4$, 1.2 ; KH_2PO_4, 1.2 ; $NaHCO_3$, 20 ; EDTA, 0.016 ; Glucose, 11.1 and $CaCl_2$, 2.5 ; pH 7.4) previously filtered (0.2 µm filter funnel) and aerated with 95% O_2- 5% CO_2 gas mixture. The heart is cannulated through the aorta in order to allow a retrograde perfusion at a constant flow rate of 12 ml/min, according to the Langendorff method (Skrzypiec-Spring et al., 2007). To assess left ventricular function, a water-filled latex balloon was inserted into the left ventricle through the mitral valve. An equilibration period of 30 min was required to ensure the stability of the parameters recorded before any molecule addition. Left ventricular developed pressure (LVDevP) was determined as the difference between left ventricular systolic pressure and left ventricular end-diastolic pressure. Coronary vasodilation was determined by perfusion pressure variation. Each parameter was recorded initially and after the addition of increasing concentrations of isoproterenol (a non-selective β-adrenoceptor agonist (0.1 nM- 1µM)).

142 All the parameters recorded were analysed by LabChart ®Pro software (V7, ADInstruments,
143 France).

144 2.5.Vascular reactivity experiments

145 Immediately after the sacrifice, thoracic aorta was rapidly removed, dissected, cleaned of fat
146 and connective tissue and cut into rings (2-3 mm long). Thoracic aortic rings were then
147 suspended on stainless-steel hooks in individual organ baths (Emka Technologies, Paris,
148 France), containing 10 ml of *Krebs-Henseleit* solution heated to 37°C and continuously
149 aerated with a 95% O_2 and 5% CO_2 gas mixture. Thoracic aortic rings were progressively
150 stretched to a resting tension of 2g. Isometric tension was detected using isometric force
151 transducers of a myograph (Emka Technologies, Paris, France) and recorded by data
152 acquisition software (iOX, Paris, France). Briefly, after 1h of equilibration at a resting tension
153 of 2g, the endothelium viability was verified by the observation of at least 60% relaxation to
154 acetylcholine (1µM) in thoracic aortic rings after phenylephrine (1µM, a selective α1-
155 adrenoceptor agonist) precontraction (Sauvaget et al., 2010). Cumulative concentration-
156 response curves (CCRCs) to phenylephrine (1nM-10µM), acetylcholine (1nM-10µM) and
157 sodium nitroprusside (a nitric oxide donor, 0.1nM- 0.1µM) were constructed. To evaluate the
158 role of inducible nitric oxide synthase (iNOS) in aortic contraction, Thoracic aortic rings were
159 incubated with aminoguanidine (100µM, an iNOS inhibitor) for 30 min. The agonist
160 maximum response and sensitivity were determined by E_{max} and $pD_2 = -\log EC_{50}$ values
161 respectively.

162 2.6.Immunofluorescence and quantification of iNOS expression

163 Thoracic aortic rings were fixed with 4% paraformaldehyde in PBS for 4 h at 4°C. After 3
164 washings in PBS, the aortic rings were incubated in PBS containing 20% sucrose for one
165 night. Then, they were embedded in tissue Tek OCT medium ®(Sakura, USA), frozen by

immersion in liquid isopentane and conserved at -80°C. Frozen section (10 µm) were permeabilized with 0.5 % Triton 100X in PBS for 5min and treated with a PBS solution containing 0.5% Triton 100X and 2% bovine serum albumin (BSA) for 1 h to block the nonspecific antigen binding.

Sections were then incubated with a rabbit polyclonal antibody against iNOS (1 :100 in the blocking buffer, Abcam, Cambridge, United Kingdom) for one night. After 5 washings of 3 min each in PBS, the sections were incubated with secondary antibody, AlexaFluor 555-conjugated donkey anti-rabbit (1 :300, life Technologie, Saint Aubin, France) for 1 h at room temperature. The slides were covered with mounting medium (Mowiol, Calbiochem, San Diego, CA, USA) and coverslip before being viewed using a spectral confocal microscope (Zeiss LSM 780, Zeiss, France). 488 nm argon laser line was used to observe elastic lamina autofluorescence while 561 nm solid state laser line was used for AlexaFluor 555 detection (iNOS immunolabellings). Image analysis was performed to evaluate iNOS expression level in the thoracic aorta of all rats by using Fiji Software. Mean Fluorescence Intensity (MFI) values were determined from 5 different fields of immunolabeled thoracic aortic sections in each group. The same threshold value was applicated on the sum intensity projections obtained from z stacks in each section. Finally, the MFI was reported to total area of analysed section.

2.7.cGMP levels in thoracic aorta, heart and epididymal fat samples

Quickly after the sacrifice, the remaining thoracic aorta, part of the heart and epididymal fat were frozen in liquid nitrogen to avoid cGMP degradation and were stored at -80°C. The cGMP content was measured colorimetrically using an immunoenzymatic assay kit (Cayman Chemical Company). A spectrophotometer at 405nm was used to read absorbance. The mean value was calculated from duplicate measurements of each sample and related to total cell

protein levels previously measured using a protein assay reagent kit (micro BCA-Pierce) (Kanso et al., 2014).

2.8.Drugs

Phenylephrine hydrochloride, acetylcholine chloride, sodium nitroprusside and isoproterenol were obtained from Sigma-Aldrich (Saint Quentin-Fallavier, France) and sodium pentobarbital solution from Ceva Santé Animale (Libourne, France). BAY 41-2272 was provided from Bayer (La Garenne-Colombes, France). All drugs were prepared in distilled water.

2.9.Statistical analysis

All the results were expressed as mean ± S.E.M of n experiments where n represents the number of rats. The results were compared using a One-way ANOVA followed by Tukey *post-hoc* test when needed. Statistical analysis were performed using GraphPad PRISM ® software version 5.

CCRCs were compared using either a non-linear mixed effect (NLME) model for complete curves or a linear mixed effect (LME) model for incomplete curves on R software (Thorin et al. 2010). $P<0.05$ was considered statistically significant.

3. Results

3.1. Effect of CD and BAY 41-2272 treatment on body weight and metabolic parameters

As shown in Table 1, at the end of the feeding period, body weight gain increased significantly more among SHR fed a CD diet than among those fed a normal diet ($P<0.001$). This weight gain was correlated to a significant increase in abdominal circumference ($P<0.001$) (Fig. 1A.1B) and epididymal fat ($P<0.01$). In this study, we examined the effect of long-term treatment with BAY 41-2272 on body weight in rats fed with CD. BAY 41-2272

213 treatment prevented excessive weight gain and increase in abdominal circumference and was
214 well tolerated by SHR as well. Moreover, CD feeding induced a rise in SBP compared to the
215 control SHR ($P<0.01$) (Table 1). Similar results were observed for DBP. The elevation in both
216 SBP and DBP was significantly attenuated by BAY 41-2272 treatment ($P<0.001$). The plasma
217 triglycerides level was significantly increased in CD-fed rats ($P<0.05$), whereas total
218 cholesterol level was reduced compared to control rats. Plasma triglycerides levels remained
219 elevated in CD-fed group treated with BAY 41-2272. However, no significant differences in
220 glucose and insulin levels among groups were noticed (Table 1).

221 Table 1: Effect of CD with or without BAY 41-2272 treatment on metabolic disorders

	Control	**CD**	**CD + BAY 41-2272**
Body weight gain (g)	145±5.13	248±9.23 [c]	216±6.11 [d]
Abdominal circumference (cm)	15.48±0.29	18.07±0.17 [c]	17.46±0.14
Epididymal fat (g)	2.33±0.08	4.41±0.53 [b]	4.03±0.47
Systolic blood pressure (mmHg)	164.4±2.2	180.0±2.6 [b]	131.6±3.7 [e]
Diastolic blood pressure (mmHg)	127.5±2.8	151.6±2.4 [c]	106.9±3.2 [e]
Triglycerides (g/l)	0.79± 0.08	1.34± 0.16 [a]	1.37± 0.17
Total cholesterol (g/l)	0.85± 0.04	0.62± 0.06 [b]	0.75±0.04
Fasting glycemia (mg/dl)	103.60± 2.48	107.30± 4.52	96.71 ± 3.43
Fasting insulinemia (µUI/ml)	9.21± 1.32	11.83± 2.52	9.87 ± 2.37

222

All values are mean ± S.E.M. [a] P<0.05, [b] P<*0.01*, [c] P<*0.001 vs* Control group, [d] P<0.05, [e] P<0.001 vs CD group; ANOVA followed by the Tuckey's multiple comparison post-hoc test / NLME model, n=14-16 animals per group. CD, Cafeteria Diet

Glucose tolerance was evaluated by intraperitoneal administration of glucose (1g/kg) to all groups. CD feeding enhanced elevation of glucose blood level in comparison to the standard chow diet (Fig. 2A). The area under the curve (AUC) was higher in the CD-fed group than in the control group (P<0.001, Fig.2B). However, long-term treatment with BAY 41-2272 significantly improved glucose tolerance in CD-fed rats (P<0.001, Fig. 2A,2B).

3.2.Isolated heart data

To examine the effects of long-term treatment with BAY 41-2272 during CD feeding, cardiac contractility and coronary perfusion pressure were evaluated using an isolated Langendorff heart preparation. As shown in Table 2, both LVDevP and coronary perfusion pressure did not differ between rats from the 3 groups at the basal level.

Table 2: Baseline cardiac parameters

	Control	**CD**	**CD + BAY 41-2272**
LVDevP (mmHg)	77.04± 8.22	60.55± 3.72	67.04± 5.20
Coronary perfusion pressure (mmHg)	25.88± 1.40	23.73 ± 3.29	22.45± 5.84

The hearts were perfused with aerated Krebs-Henseleit solution as previously described. LVDevP, Left ventricular developed pressure. CD, Cafeteria Diet. n=6 animals/group. Values are expressed as mean ± S.E.M

Moreover, in order to determine the effects of BAY 41-2272 on β-adrenergic response, we evaluated cardiac function of the 3 groups under stimulation of increasing concentrations of isoproterenol. β-adrenoceptor stimulation induced a marked increase in LV contractility

(determined by LVDevP and d*P*/d*t* (max)) in control rats. This positive inotropic effect was significantly reduced in CD-fed group ($P < 0.001$) (Fig. 3A,3C). Similarly, LV relaxation (d*P*/d*t* min) was also significantly depressed after isoproterenol stimulation in CD-fed group compared to the control group ($P<0.01$) (Fig. 3D). Thus, in our rat model of metabolic syndrome, basal cardiac function was preserved but the inotropic and lusitropic effects in response to β-adrenoceptor stimulation were altered suggesting an impairment in the β-adrenoceptor signalling in this model. However, long-term treatment with BAY 41-2272 significantly restored the isoproterenol-induced lusitropy and the inotropy parameters. These findings revealed that *in vivo* long-term sGC stimulation in CD-fed rats improved cardiac systolic and diastolic functions (Fig. 3A,3C.3D). In parallel, isoproterenol-induced coronary vasodilation was higher in control group than in CD-fed group ($P<0.05$). This parameter was also restored in CD group treated with BAY 41-2272 (Fig. 3B).

3.3.Vascular reactivity

Next, aortic reactivity was also evaluated. CCRCs to phenylephrine were then constructed in aortic rings from all groups. The maximal contractile response (E_{max}) to phenylephrine, in aortic rings from CD-fed group was significantly lower than that in the control group ($P<0.01$) (Fig.4A, Table 3). However, this response was normalized after adding an iNOS inhibitor (Aminoguanidine, 100µM) in the bathing solution (Fig.4B, Table 3). These findings suggest an iNOS-dependent excessive NO release in the CD-fed group. On another side, BAY 41-2272 treatment completely restored the phenylephrine- concentration response curve in CD-fed rats with significant increase in the maximal force of contraction (Fig. 4A, Table 3).

Table 3: pD_2 and E_{max} values of phenylephrine in the presence and in the absence of aminoguanidine in aortic rings isolated from all groups

	Control	CD	CD + BAY 41-2272
	Phe		
E_{max} (g)	4.16± 0.07 [a]	3.77± 0.09	4.30± 0.07 [b]
pD_2	7.27± 0.04	7.30± 0.05	7.65± 0.06
	Phe + AMN		
E_{max} (g)	4.57± 0.21	4.46± 0.29 [c]	5.07± 0.10
pD_2	7.28± 0.23	7.30± 0.08	7 .59± 0.16

Values are mean ± S.E.M. n=14-16 animals/group. [a] P<0.01 vs CD, [b] P <0.001 vs CD, [c] P<0.001 vs CD without AMN pretreatment determined by NLME model. Phe, Phenylephrine, AMN, Aminoguanidine, CD, Cafeteria diet

3.4.Quantification of iNOS expression in thoracic aorta

To test the hypothesis of a possible involvement of iNOS in the altered response to phenylephrine in CD-fed rats, immunofluorescence labelling was performed to determine the iNOS expression in thoracic aorta isolated from all groups. Our results did not show any significant difference between the control and CD-fed groups. However, the iNOS enzyme protein expression was significantly higher in CD-fed rats treated with BAY 41-2272 ($P<0.05$) (Fig. 5A, 5B).

3.5.Endothelium-dependent and independent relaxations

In the present study, we evaluated the effects of both metabolic syndrome and long-term treatment with BAY 41-2272 on endothelium-dependent vascular relaxation. Our results showed that acetylcholine-induced endothelium- dependent relaxation was similar in aortic rings from control and CD-fed group (Fig. 6A). This finding indicates that endothelial function was not altered in our model of metabolic syndrome. Furthermore, maximal relaxation in response to acetylcholine was not enhanced in aortic rings isolated from CD-fed group treated with BAY 41-2272. Similarly, no significant difference in endothelium-independent relaxation in response to SNP among the groups was noted (Fig. 6B).

3.6. Intracellular cGMP content in thoracic aorta, heart and epididymal fat samples

To further explore sGC-cGMP signalling pathway in our experimental conditions, we assessed intracellular cGMP content in thoracic aorta, heart and epididymal fat samples isolated from all groups. The results showed that compared to the CD-fed group, the cGMP content was slightly but not significantly increased in CD- fed group treated with BAY 41-2272 both in cardiac and vascular tissue. On the other hand, the cGMP content was significantly higher in epididymal fat samples of CD-fed group treated with BAY 41-2272 in comparison to the CD untreated group (Table 4).

Table 4: intracellular cGMP level in thoracic aorta, heart and epididymal fat samples

cGMP (pmol/mg protein)	Control	CD	CD + BAY 41-2272
Thoracic aorta	1.20± 0.23	0.89± 0.12	1.40± 0.27
Heart	1.10± 0.12	1.25 ± 0.34	1.67± 0.16
Epididymal fat	0.33±0.07	0.16±0.04	1.15±0.28 [a]

Values are expressed as means ± S.E.M. CD, Cafeteria diet. n=8 animals/group. [a] P <0.01vs CD. ANOVA followed by the Tuckey's multiple comparison post-hoc test. CD, Cafeteria Diet

4. Discussion

In the present study, we showed that chronic CD feeding of SHR, induced abdominal obesity, hypertriglyceridemia, glucose intolerance and arterial hypertension, which are the main hallmarks of metabolic syndrome. These findings are consistent with those of La Russa et al. (2019) who demonstrated that CD is the most appropriate regime to induce severe obesity, glucose intolerance, insulin resistance, and high plasma triglyceride levels in rodents. However, they are partially inconsistent with the findings of Miesel et al. (2010) who reported that insulin resistance in SHR was more pronounced over the feeding period. It is well documented that SHR are already hypertensive and insulino-resistant (Reaven and Chang, 1991). Moreover, it is probable that CD-fed group presented insulin resistance, even if insulin levels were not modified, since obesity was associated to reduced glycemic tolerance in that group (Oliveira Junior et al., 2010).

One of the main findings of the present study is that long-term treatment with BAY 41-2272 reduced body weight gain and abdominal fat and improved glucose tolerance in CD-fed rats in comparison to CD untreated rats. These results seem to be more related to increased energy expenditure than to decreased energy intake since food consumption between untreated and treated groups was not statistically different (data not shown). These results are consistent with our previous work which showed that cGMP pathway activation through PDE5 inhibition, prevented weight gain in SHR fed a CD (Doghri et al., 2019). They are also in agreement with the findings of Mitschke et al. (2013) who demonstrated that short-term treatment with sildenafil in mice, increased the uncoupling protein-1 (UCP-1) expression and promoted browning of white adipose tissue which is considered a primary site of energy expenditure (Haas et al., 2009). Moreover, a recent study showed that pharmacological stimulation of sGC induced weight loss and improved the metabolic phenotype in mice with diet-induced obesity by enhancing brown adipocytes differentiation (Hoffmann et al., 2015). Increasing evidence suggests that cGMP pathway modulation may regulate energy balance via mechanisms that involve thermogenesis, by promoting mitochondrial biogenesis and increasing the abundance of UCP-1(Kim GW et al., 2014).

Although several mechanisms have already been described to better understand the metabolic syndrome-induced cardiac dysfunction (Ilkun and Boudina, 2013; Tune et al., 2017), little information is available on the β-adrenergic system changes during the metabolic syndrome in SHR. The significant decrease in both inotropic and lusitropic effects of β-adrenoceptor stimulation observed in CD-fed group could be related to either alteration in intracellular calcium handling (Lima-Leopoldo et al., 2011; Nevelsteen et al., 2013) or to the down-regulation of β_1- and β_2- adrenoceptors (Jiang et al., 2015). It is well known that sympathetic nervous system plays a crucial role in maintaining cardiovascular homeostasis (Manolis et al., 2014). In this regard, Li et al. (2015) have demonstrated an enhanced sympathetic activity in

SHR compared to normotensive rats. Moreover, several line of evidence show a link between metabolic syndrome and sympathetic overactivity (Thorp and Schlaich, 2015). Therefore, it is likely that sustained sympathetic overstimulation associated with metabolic syndrome may contribute to the downregulation of myocardial β- adrenoceptors. On another side, previous research has reported that the sympathetic overactivation associated with the development of metabolic syndrome, resulted in vascular hyporeactivity to the α_1-adrenoceptor stimulation in rats (Battaut et al., 2018). These observations are in line with data from our study. In the present study, CD-fed group showed a lower vasoconstrictor response to the α_1-adrenceptor agonist, phenylephrine compared to their counterparts in the control group. Reduced aortic contractility was also reported in aortic rings from other animal models of metabolic syndrome such as obese zucker rats (Vendrame et al., 2014) and high sugar-fed mice (Silva et al., 2016). However, one alternative explanation for such a result is the increase in iNOS-induced NO production in CD-fed group. This hypothesis is supported by the fact that addition of the iNOS inhibitor, aminoguanidine completely normalized the response to phenylephrine in this group. Nonetheless, immunofluorescence assay did not show any significant difference in iNOS expression in thoracic aorta between the control and CD-fed groups. Data from literature concerning the iNOS expression modification in rat aorta during metabolic syndrome are divergent (Araujo et al., 2018; Cebova et al., 2018). The possible mechanisms explaining these divergent findings remain unclear and may be related to type of diet and animal model used. Thus, further studies are needed to clarify the mechanisms underlying these discrepancies.

A major finding of this study is that the sCG stimulator BAY 41-2272, administered orally to CD-fed SHR at the dose of 5 mg/kg for 12 weeks was able to attenuate significantly both systolic and diastolic hypertension. Our results are in agreement with previous studies. Geschka et al. (2011) showed that sGC stimulation by riociguat at dose of 3mg/kg for 14

weeks were sufficient to decrease markedly systemic hypertension and to improve survival in Dahl salt-sensitive rats. Furthermore, a study established by Stasch et al. (2001) demonstrated that oral administration of BAY 41-2272 (1-10 mg/kg) resulted in a strong decrease in blood pressure in a low-NO rat model of hypertension. In is not unreasonable to postulate that the BAY 41-2272 lowering blood pressure effect could play a role in the change of cardiovascular reactivity observed in our study. Typically, the majority of antihypertensive drugs may exert simultaneously both specific local and systemic effects; and it is not easy to distinguish the drug-induced reduction of blood pressure from any local effect that may occur at the cellular level. Ideally sub-antihypertensive doses of BAY 41-2272 should be tested in order to accurately assess the effect of BAY 41-2272 on cardiovascular reactivity independently of the change in blood pressure.

In addition to lowering blood pressure, long-term treatment with BAY 41-2272 improved β-adrenoceptor responsiveness and restored the adrenoreceptor-mediated vasoconstrictive response in CD-fed rats. There is accumulating evidence that sGC stimulators possess anti-fibrotic, anti-inflammatory and antioxidant properties (Tobin et al., 2018). Moreover, a study conducted by Ferron et al. (2019) showed that improved β-adrenergic responsiveness in high sugar-fat diet fed rats would be attributed to reduced oxidant status. In light of our results, we suggest that improvement in the adrenergic response observed in CD-treated rats may involve antioxidant properties of BAY 41-2272 in addition to its vasodilator potential. Unfortunately, we were not able to get sufficient blood samples to analyze the oxidative status as we needed to keep a good viability of the heart for *ex vivo* cardiac reactivity. Another plausible explanation for this finding is a possible cross regulation between cyclic adenosine monophosphate (cAMP)- and cGMP- mediated signalling pathways. It has been previously shown that increasing levels of cGMP suppress cAMP hydrolysis rate by phosphodiesterase 1,2 and 3, leading to an amplified cAMP signalling (Zhao et al., 2015). This suggests that

long-term treatment with BAY 41-2272 improves β-adrenergic responsiveness probably indirectly through a mechanism that may involve isoenzymes phosphodiesterase regulation.

According to other reports (Vendrame et al., 2014; Lyoussi et al., 2018), we showed that acetylcholine-induced endothelium- dependent relaxation was similar in aortic rings from control and CD-fed group. However, this result contrasts with other findings describing a reduced endothelium-dependent relaxation in SHR (Anishchenko et al., 2015) and in other animal models of metabolic syndrome (El-Bassossy et al., 2014; Bhatta et al., 2017). A probable hypothesis to explain this divergence is that a compensatory mechanism likely involving a role of NO was developed in CD-fed rats to offset the effects of arterial hypertension. This hypothesis is consistent with findings of Berenyiova et al. (2018), who showed that SHR develop adaptative mechanisms by preserving NOS activity level in order to fight chronic NO deficiency. In addition, this is in line with the lack of difference between groups concerning intracellular cGMP content in thoracic aorta observed in our experimental conditions. It is widely documented that resistant arteries play an important role in the regulation of blood pressure in SHR (Yu et al., 2016) and are less dependent on NO than thoracic aorta (Lyoussi et al., 2018). A complementary study of the vascular reactivity in resistance arteries would therefore be interesting to better assess the effects of CD and BAY 41-2272 treatment.

In conclusion, the present study demonstrated that long-term treatment with BAY 41-2272 prevented excessive weight gain, markedly attenuated arterial hypertension and improved cardiovascular reactivity in CD-induced metabolic syndrome in SHR. These results need deeper investigations to assess whether BAY 41-2272 might represent a promising potential candidate in the management of the metabolic syndrome and associated cardiovascular alterations.

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Conflict of interest

The authors declare that no competing interests exist

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Figure captions

Fig. 1. Follow up of body weight gain (A) and abdominal circumference (B) during 12 weeks of diet. CD feeding significantly increase body weight gain and abdominal circumference in comparison to the standard chow diet ($P < 0.001$). On the other hand, long-term treatment with BAY 41-2272 limited excessive weight gain only at the 12^{th} week of the treatment ($P<0.05$ vs CD) and tended to prevent increase in abdominal circumference in CD-fed group. Data are expressed as mean ± S.E.M (n=14-16 rats/group). *$P<0.001$ vs Control determined by LME model.

Fig. 2. Blood glucose concentrations (A) and AUC (B) of intraperitoneal glucose tolerance test were measured at the end of experimental protocol in all groups. Long-term treatment with BAY 41-2272 improved glucose tolerance in CD-fed rats. ***$P<0.001$ vs Control, CD + BAY 41-2272 by One-Way ANOVA followed by Tukey *post hoc* test (n=14-16 rats/group).

Fig. 3. Cardiac response to isoproterenol in CD-fed rats and CD-fed rats treated with BAY 41-2272. The contractile function was evaluated by measuring the increase in left ventricular developed pressure (LVDevP) (A) and time derivative of pressure during contraction (dP/dt max) in response to isoproterenol (C). Coronary vasodilation was assessed through coronary perfusion pressure variation (B). The diastolic function of the heart was evaluated by measuring the increase in time derivative of pressure during relaxation (dP/dt min) (D). Each value represents the mean ± S.E.M (n=6 rats/group). *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs CD determined by LME model.

Fig. 4. Cumulative-concentration response curves to phenylephrine in thoracic aortic rings isolated from control rats, CD-fed rats and CD-fed rats treated with BAY 41-2272. Contractile response to phenylephrine in the absence (A) or presence of aminoguanidine (100 µM) (B).

662 Each value corresponds to the mean ± S.E.M (n=14-16 rats/group). **P<0.01, ***P<0.001 vs
663 CD determined by NLME model.

664 Fig. 5. Effects of CD and long-term treatment with BAY 41-2272 on iNOS enzyme protein
665 expression in thoracic aorta. (A) Fluorescence confocal microscopy of iNOS (red
666 fluorescence, λexc 561 nm) immunodetected in thoracic aorta (elastin with green
667 fluorescence, λexc 488 nm), scale bar 50 µm. Control group (n = 4), CD-fed group (n = 3)
668 and CD-fed group treated with BAY 41-2272 (n=3). (B) Values were represented as mean ±
669 S.E.M of mean fluorescence intensity (U.I.). *P<0.05 vs Control /CD group determined by
670 One Way ANOVA.

671 Fig. 6. Effects of chronic CD feeding and long-term treatment with BAY 41-2272 on
672 endothelium-dependent and independent relaxations in thoracic aorta. Cumulative
673 concentration response of acetylcholine-induced relaxation (A). Cumulative concentration
674 response curve to sodium nitroprusside (SNP) for the endothelium-independent relaxation
675 (B). values are expressed in percentage of the precontraction. Each value represents the mean
676 ± S.E.M. Comparisons were performed using NLME model.

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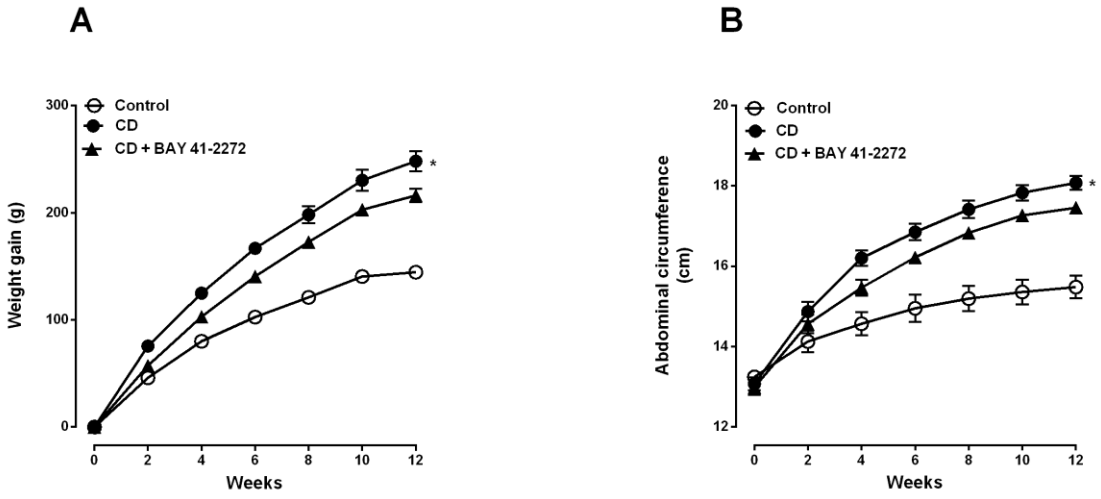
A
B
Control
CD
CD + BAY 41-2272
Weight gain (g)
Abdominal circumference (cm)
Weeks
*
*

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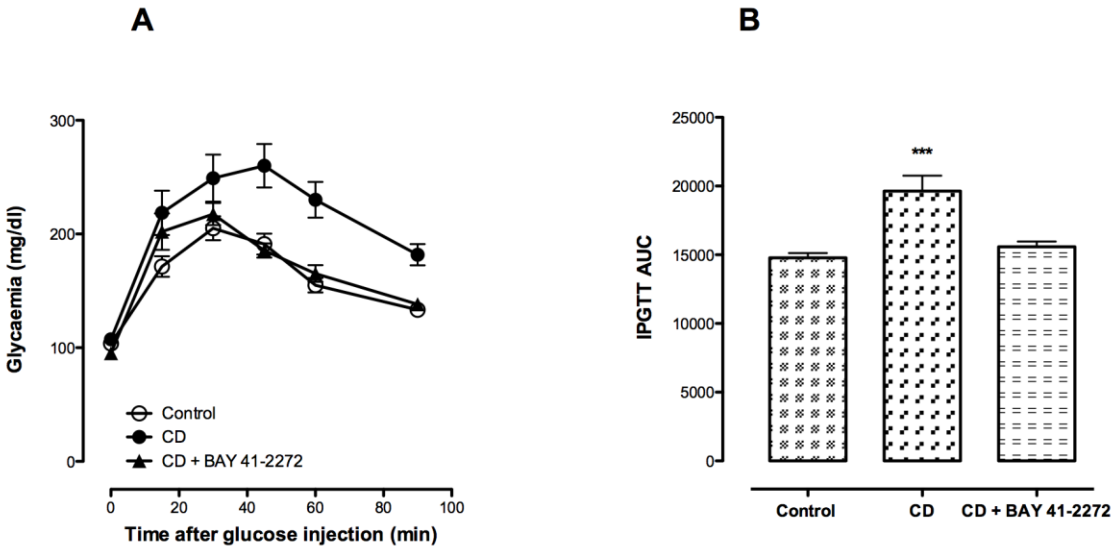
A
B
Glycaemia (mg/dl)
300
200
100
0
Control
CD
CD + BAY 41-2272
0
20
40
60
80
100
Time after glucose injection (min)
IPGTT AUC
25000
20000
15000
10000
5000
0
***
Control
CD
CD + BAY 41-2272

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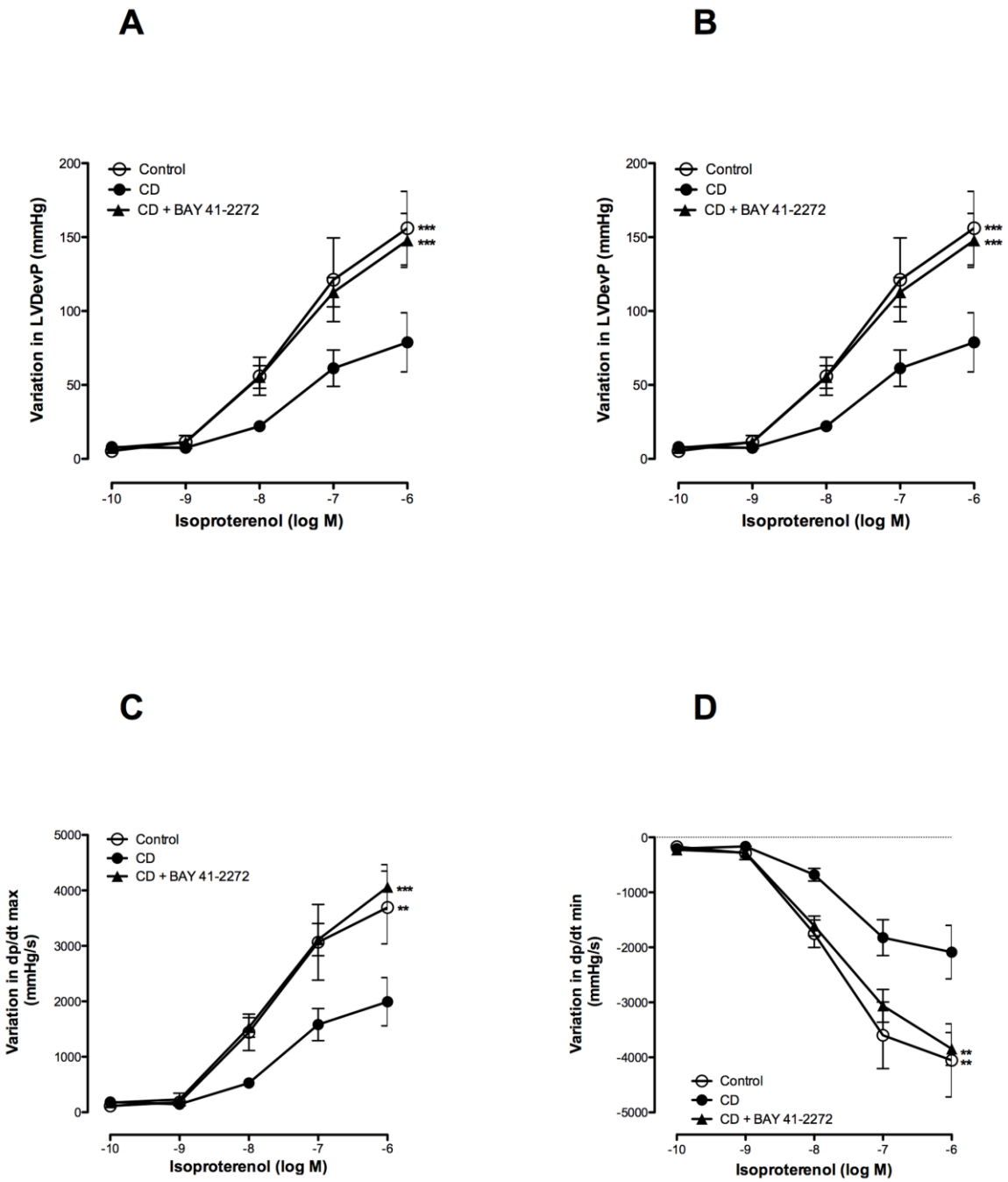
A
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Control
CD
CD + BAY 41-2272
Variation in LVDevP (mmHg)
Isoproterenol (log M)
Variation in dp/dt max (mmHg/s)
Variation in dp/dt min (mmHg/s)
***
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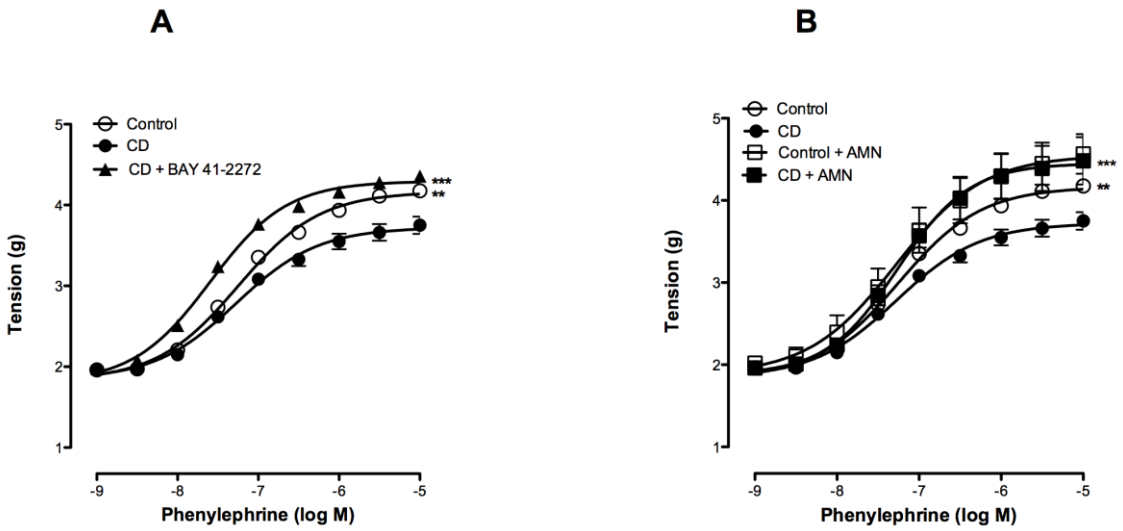
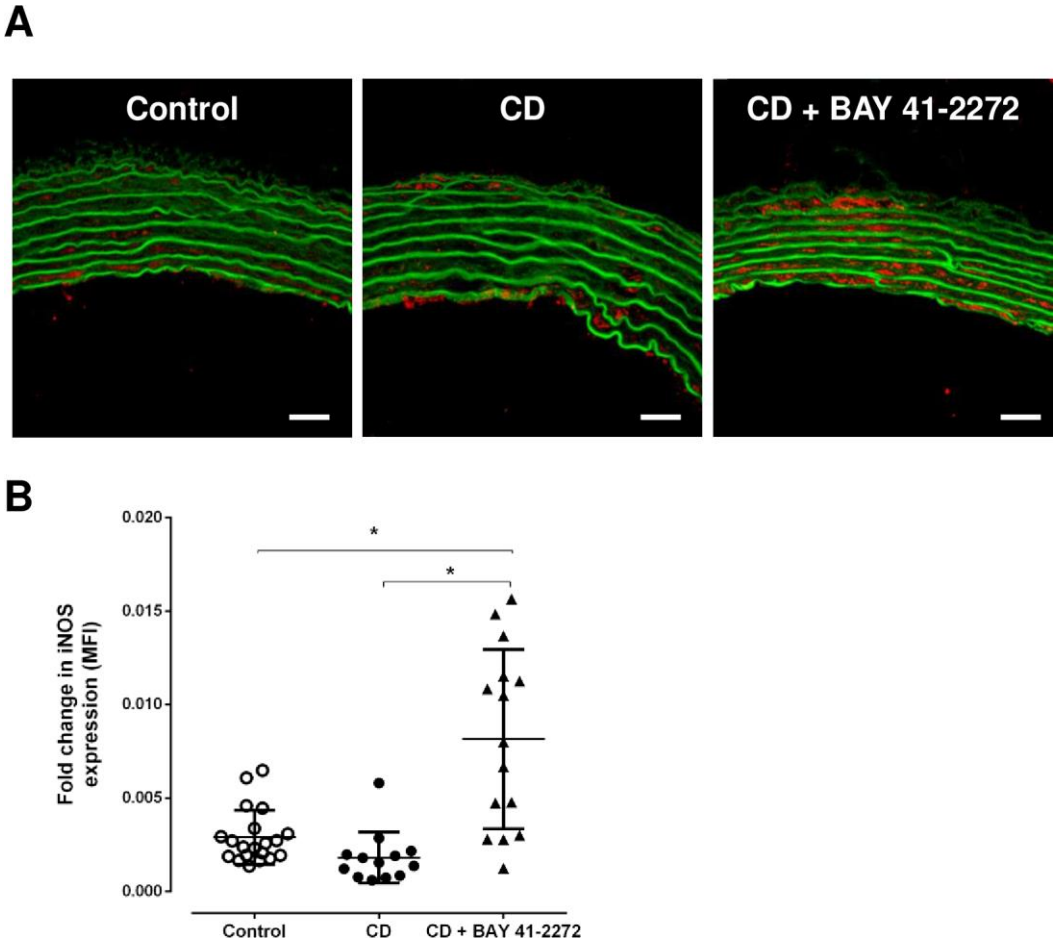
682

683


A
Control
CD
CD + BAY 41-2272
***
**
Tension (g)
Phenylephrine (log M)
B
Control
CD
Control + AMN
CD + AMN
***
**
Tension (g)
Phenylephrine (log M)

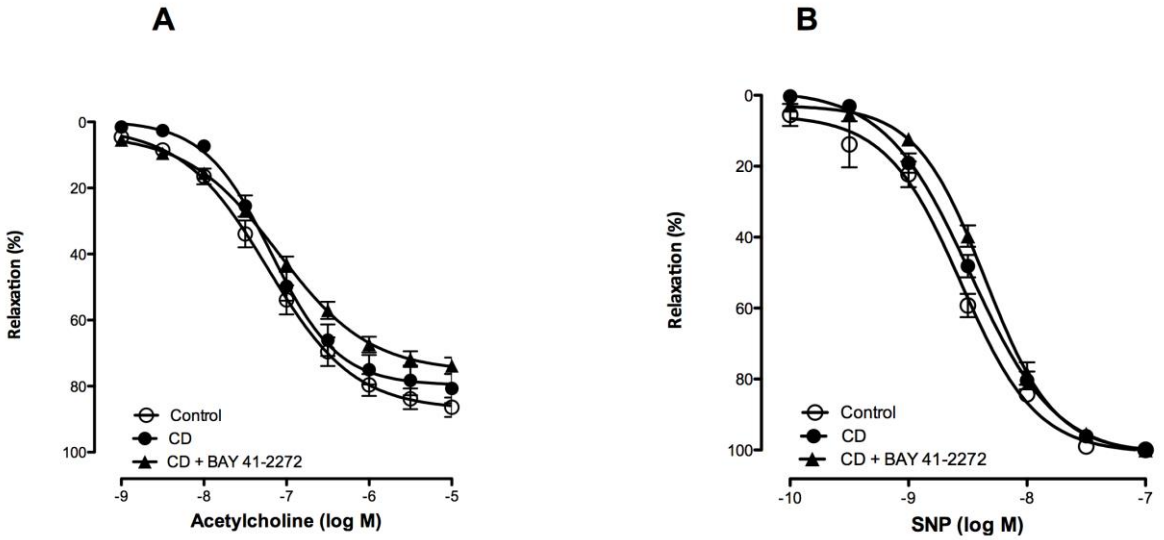
684

685


A
Control
CD
CD + BAY 41-2272
B
Fold change in iNOS expression (MFI)
0.020
0.015
0.010
0.005
0.000
*
*
Control
CD
CD + BAY 41-2272

686

687


A
Relaxation (%)
0
20
40
60
80
100
Control
CD
CD + BAY 41-2272
-9
-8
-7
-6
-5
Acetylcholine (log M)
B
Relaxation (%)
0
20
40
60
80
100
Control
CD
CD + BAY 41-2272
-10
-9
-8
-7
SNP (log M)

688