Reactivity of the aorta and mesenteric resistance arteries from the obese spontaneously hypertensive rat: effects of glitazones

Yolanda Mendizábal, Silvia Llorens, and Eduardo Nava

Department of Medical Sciences, University of Castilla-La Mancha, School of Medicine and Regional Centre for Biomedical Research, Albacete, Spain

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Mendizábal Y, Llorens S, Nava E. Reactivity of the aorta and mesenteric resistance arteries from the obese spontaneously hypertensive rat: effects of glitazones. Am J Physiol Heart Circ Physiol 301: H1319–H1330, 2011. First published July 22, 2011; doi:10.1152/ajpheart.01280.2010.—The obese spontaneously hypertensive rat (SHROB) is a model of metabolic syndrome in which, to our knowledge, vascular function has never been studied. The actions of insulin sensitizers (glitazones) on vascular function have not been analyzed either. Our purpose was to characterize microvascular and macrovascular responses of the SHROB and to study the effects of glitazones on these responses. The reactivity of mesenteric resistance arteries (MRAs) and the aorta from SHROBs and control rats to cumulative concentrations of phenylephrine, ACh, and sodium nitroprusside (SNP) was myographically analyzed. Some animals were orally treated with rosiglitazone (3 mg·kg⁻¹·day⁻¹, 3 wk), and myography was performed. Phenylephrine, ACh, and SNP dose-response curves were impaired to different extents in arteries of SHROBs. Incubation with N-nitro-L-arginine methyl ester caused little effects on phenylephrine and ACh curves in MRAs but enhanced phenylephrine contractions and abolished ACh-induced relaxations of aortae. Incubation with indomethacin reduced phenylephrine reactivity and improved ACh-induced relaxations of all vessels studied. NS-398 and tempol increased relaxations to ACh of MRAs. Incubation with pioglitazone or rosiglitazone (both 10⁻³ M) or oral treatment with rosiglitazone improved, to different extents, ACh and SNP curves in all vessels. Glitazone incubation diminished aortic ACh sensitivity. The release of thromboxane A₂ and PGl₂ metabolites (thromboxane B₂ and 6-keto-PGF₁α) was analyzed. ACh increased the MRA release of thromboxane B₂ from SHROBs but not control rats, and the former was prevented by rosiglitazone coincubation. In contrast, in aortae, ACh failed to alter the release of metabolites, and rosiglitazone treatment increased that of 6-keto-PGF₁α. Thus, SHROBs displayed microvascular and macrovascular dysfunction. MRAs, but not aortae, of SHROBs revealed an impaired endothelial nitric oxide pathway, whereas both, but especially MRAs, displayed an impaired cyclooxygenase pathway. Glitazones elicited beneficial effects on macrovascular and, especially, microvascular function of SHROBs.

disorders that frequently take place in metabolic syndrome are macrovascular disease (macroangiopathy) and microvascular disease (microangiopathy) (6, 10, 25, 28). The amount of epidemiological research on metabolic syndrome is quite abundant (6, 20, 25). However, vascular research in animal models of metabolic syndrome is relatively limited, despite the high relevance of blood vessel disease in type II diabetes mellitus (32).

The obese spontaneously hypertensive rat (SHROB) is a rat strain of spontaneous hypertension breeding origin that suffers a nonsense mutation of the leptin receptor gene (7). These rats display all the symptoms of metabolic syndrome and are generally regarded as an adequate animal model of this disease (2). Remarkably, there is a complete lack of studies on vascular and endothelial function in this animal model. Therefore, the major aim of the present work was to characterize the vascular responses of SHROBs compared with those of healthy Wistar-Kyoto (WKY) rats.

Macroangiopathy and microangiopathy are potential vascular complications of metabolic syndrome patients (6, 25). In addition, macrovessels and microvessels play differential roles in diabetic conditions (12). Therefore, we focused our study on two kinds of arteries: a large conduction vessel (the aorta) and on resistance arteries [mesenteric resistance arteries (MRAs)]. We analyzed the responses of these two vessels to adrenergic stimulation, sodium nitroprusside (SNP), and ACh (endothelium-independent and –dependent relaxation, respectively). In addition, we tested the effects of nitric oxide (NO) synthase and ROS inhibition as well as unspecific and specific inhibitors of the synthesis or receptor of various prostaglandins so as to determine the respective roles of these endothelial paracrine mediators.

Glitazones are insulin sensitizers used in type II diabetes mellitus that confer beneficial actions on most or every symptom of metabolic syndrome beyond the ordinary effects on glucose and lipid metabolism (23, 24). For this reason, they are considered an adequate tool for metabolic syndrome research (15). Cardiovascular disorders associated with insulin resistance are not an exception to all this. Indeed, glitazones are able to lower blood pressure and decrease vascular smooth muscle contractility (24). The mechanisms underlying these abilities are unclear. In this regard, we (16) have recently shown in the spontaneously hypertensive rat (SHR) that, provided cyclooxygenase is inhibited, these drugs do improve aortic endothelial function. Vascular pharmacology studies with glitazones have never been attempted in the SHROB. Therefore, the second aim of this work was to study the effects of two glitazones that have been extensively used in the clinic, pioglitazone and rosiglitazone, on vascular function, both macrovascular and microvascular, of the SHROB.

Address for reprint requests and other correspondence: E. Nava, Dept. of Medical Sciences, Univ. of Castilla-La Mancha School of Medicine and Regional Centre for Biomedical Research, Albacete 02006, Spain (e-mail: eduardo.nava@uclm.es).

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MATERIAL AND METHODS

Preparation of the Animals and Blood Pressure Determination

We used 16- to 25-wk-old male WKY rats and SHROBs obtained from Charles River Laboratories (Barcelona, Spain, and Wilmington, DE, respectively). Animals were housed in ventilated racks located in a one-way airflow system room (temperature: 20–22°C; 12:12-h light-dark cycle; humidity: 45%). All procedures were approved by and carried out with the permission of the Animal Experimentation Ethics Committee of the University of Castilla-La Mancha and in accordance with the Declaration of Helsinki and Spanish Real Decreto 1201/2005 on Protection of Animals Utilized for Experimentation and Other Scientific Purposes. Systolic blood pressure and heart rate were determined in conscious rats by means of a tail cuff (always from 12:00 and 19:00 hours). Animals were introduced into size-adapted restrainers and kept in a quiet and warm environment for 1 h. This procedure was repeated for 2–3 days to familiarize rats with the restrainers. A rubber cuff (proximally) and a photoelectric sensor of pulsations (more distally) were placed around the tail. This sensor was connected to an amplifier (NIPREM 645, Cibertec, Madrid, Spain), and pulsations were recorded on a PowerLab recording unit (AD Instruments, Castle Hill, NSW, Australia).

Surgical Procedures, Preparation of the Tissues, and Blood Samples

Rats were fasted for at least 5 h before the procedures. On the day of the experiment, a venous blood sample was withdrawn for standard biochemical (serum) and insulin (plasma) analyses. Animals euthanized by means of CO2 inhalation underwent a laparotomy and a thoracotomy. To obtain MRAs, the intestine together with the entire mesenteric vascular bed was dissected. If the tissue was intended for analysis of prostaglandins, the vascular tissue was intended for analysis of prostaglandins, the

Preparation of Blood Vessels for Myographical Experiments

MRAs were cut in sequential 1.5- to 2-mm segments. These were immersed in myograph organ baths filled with chilled KHS. Two tungsten wires (40 μm in diameter) were carefully introduced through the arterial lumen under a microsurgery microscope and fastened with microscrews. The organ bath was then warmed to 37°C and bubbled with 95% O2-5% CO2 to provide a pH of 7.3–7.4. The segments’ length was assessed with the ocular’s microscale and stretched to the optimal circumference length (Lo) with a micrometer to obtain the maximum force performance. All these procedures were carried out with two four-channel myographs (Danish MyoTechnology, Aarhus, Denmark), and force was recorded on the above-mentioned PowerLab recording unit. Lo for each segment was obtained as follows: segments were sequentially stretched, and force was calculated. Length and force data were used to plot a wall tension–internal circumference length relationship, from which Lo was chosen following Mulvany and Halpern criteria (21). The effective luminal diameter was calculated from Lo. All calculations were carried out with the assistance of the computer software (Myonorm, Cibertec). Once stretched to Lo, a 30-min rest was allowed.

The aorta was cut into sequential 3.3-mm aortic segments and immersed in a 5-mL organ bath containing 37°C KHS bubbled as described above. Two stainless steel L-shaped pins (200 μm in diameter) were introduced through the arterial lumen under a microsurgery microscope. Isometric tension was recorded by means of the above-mentioned myographs. Preliminary experiments showed that the optimal passive tension was 2.5 g for aortic segments of both animal strains. When arterial segments were mounted in the organ bath, this tension was applied, and they were allowed to equilibrate for a period of at least 60 min before the experiments were started. Tension was readjusted when necessary, and the bath fluid was changed every 15 min. In both kinds of arteries, the reactivity of the arterial segments was checked by depolarization with high-K+ solution (K+ ± KHS). This had the same composition as KHS but contained 4.6 mM NaCl and 115 mM KCl (MRAs) or 6.96 mM NaCl and 50 mM KCl (aortae). The status of the endothelial layer was tested with a single dose of ACh (10−5 M) after phenylephrine (10−6 M). Intact vessels failing to achieve at least 60% of the maximal average relaxation were assumed to be damaged and discarded.

Vasoactive Performance of the Aorta and MRAs

The following protocols were aimed to analyze the basic vascular physiology of SHROBs compared with normotensive WKY rats.

Protocol 1: adrenoceptor-mediated contractility.

Smooth muscle contractility was tested by the study of cumulative concentrations of phenylephrine (aortae: 10−9–10−5 M and MRAs: 10−8–10−4 M). The

Table 1. Physiological and biochemical parameters of the animals used in the study

<table>
<thead>
<tr>
<th></th>
<th>WKY Rats</th>
<th>SHROBs</th>
<th>SHROBs With Oral Rosiglitazone Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Means ± SE</td>
<td>n</td>
<td>Means ± SE</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>353 ± 11</td>
<td>40</td>
<td>510 ± 7*</td>
</tr>
<tr>
<td>Systolic pressure, mmHg</td>
<td>133 ± 6</td>
<td>7</td>
<td>169 ± 4*</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>2.29 ± 0.7</td>
<td>6</td>
<td>34 ± 4*</td>
</tr>
<tr>
<td>Glucose, mg/ml</td>
<td>229 ± 15</td>
<td>10</td>
<td>300 ± 10*</td>
</tr>
<tr>
<td>Cholesterol, mg/ml</td>
<td>101 ± 4</td>
<td>10</td>
<td>303 ± 25*</td>
</tr>
<tr>
<td>Triglycerides, mg/ml</td>
<td>145 ± 15</td>
<td>10</td>
<td>492 ± 63*</td>
</tr>
<tr>
<td>HDL, mg/ml</td>
<td>70 ± 4</td>
<td>10</td>
<td>120 ± 5*</td>
</tr>
<tr>
<td>Urea, mg/ml</td>
<td>32 ± 2</td>
<td>10</td>
<td>51 ± 3*</td>
</tr>
<tr>
<td>Creatinine, mg/ml</td>
<td>0.45 ± 0.02</td>
<td>10</td>
<td>0.47 ± 0.03</td>
</tr>
</tbody>
</table>

n, Number of rats. WKY rats, Wistar-Kyoto rats; SHROBs, obese spontaneously hypertensive rats. *P < 0.01, SHROBs vs. WKY rats; ‡P < 0.05 and ‡P < 0.01, SHROBs orally treated with rosiglitazone versus control nontreated SHROBs.
roles of NO or prostaglandins in the modulation of the contraction were tested by performing the same experiment in the presence of N-nitro-L-arginine methyl ester (L-NAME; 10⁻⁴ M) or indomethacin (10⁻⁵ M).

Protocol 2: endothelium-dependent relaxation. This was tested by means of cumulative concentration-response curves of ACh (10⁻⁹–10⁻⁵ M), which was added to the myograph chamber when the last concentration of phenylephrine in protocol 1 reached a stable force. The roles of NO and prostaglandins were tested in a similar manner as in protocol 1.

Protocol 3: role of vasoactive prostaglandins in the endothelium-dependent relaxation of MRAs. The specific role of different prostanooids was tested solely in microvessels by performing the same experiment performed in protocol 2 but in the presence of either the inhibitor of cyclooxygenase-2 NS-398 (10⁻⁵ M), the thromboxane TP receptor antagonist SQ-29,548 (10⁻⁶ M), or the inhibitor of PGH₂ synthase tranycypromine (TCP; 10⁻⁵ M), which were incubated in the bath for 20 min.

Protocol 4: endothelium-independent relaxation. This was tested by means of dose-response curves to SNP. After a preconstriction with phenylephrine (aorta: 10⁻⁵ M and MRAs: 10⁻⁴ M), vessels were exposed to SNP (10⁻⁹–10⁻⁴ M) in a cumulative fashion. In some experiments, papaverine (3 × 10⁻⁴ M) was added to the organ bath at the end of the experiments after the precontraction with K⁺-KHS.

Protocol 5: role of superoxide radicals in the endothelium-dependent and -independent relaxation of MRAs. Protocol 5 was the as protocols 2 and 4, but the SOD mimetic tempol (10⁻⁴ M) was added to the organ bath 20 min before the agonists.

Acute Effects of Glitazones

The following protocols were meant to study the direct effects of glitazones on the vasoactive responses of SHROBs. Pioglitazone or rosiglitazone was added to the myograph to a concentration of 10⁻⁵ M, and vessels were incubated for 20 min.

Protocol 6: acute effect of pioglitazone and rosiglitazone on adrenoceptor-mediated contractility. Cumulative concentrations of phenylephrine (aorta: 10⁻⁹–10⁻⁵ M and MRAs: 10⁻⁸–10⁻⁴ M) were tested in the presence of pioglitazone or rosiglitazone.

Protocol 7: effect of ibidem on endothelium-dependent relaxation. Cumulative concentration-response curves to ACh were generated as in protocol 2 but in the presence of pioglitazone or rosiglitazone.

Protocol 8: effect of ibidem on endothelium-independent relaxation. Dose-response curves to SNP were generated as in protocol 4 but in the presence of pioglitazone or rosiglitazone.

Chronic Effects of Rosiglitazone

Before the initiation of the rosiglitazone treatment, several tail-cuff recordings of systolic blood pressure and heart rate were obtained on consecutive days. The day before commencement, a 0.25- to 0.3-ml venous blood sample was taken for insulin determination. The sample was withdrawn from the caudal vein of fasted rats under anesthesia [ketamine (75 mg/kg ip) and xylazine (10 mg/kg ip)]. Rats were given Avandia [rosiglitazone hydrochloride (3 mg·kg⁻¹·day⁻¹)] mixed with chow for 3 wk. This dosing has been supported by a pharmacokinetic study in rats (17). The chow was ground to powder; drug tablets were pulverized and mixed in the grinder with the chow powder. This mixture was provided to the rats in a feeder provided with a waste collector to enable an accurate daily measure of powder consumption. Body weight and chow consumption were checked on a 2- to 3-day basis to readjust the dosing.

Systolic blood pressure and heart rate (tail cuff) were assessed during 2–3 consecutive days before the cessation of the 3-wk treatment. Once this period concluded, the procedures described for acute experiments were carried out. Both aortic and mesenteric segments were tested with myograph experiments that included dose-response curves to phenylephrine, as in protocol 1, dose-response curves to Ach, as in protocol 2, and dose-response curves to SNP, as in protocol 4.

Determination of Prostaglandin Metabolite Release From Small Arteries and Aortic Segments

The dissected mesenteric vascular bed was allocated in three Eppendorf tubes containing warm KHS and bubbled as described above. The following protocols were run.

**Tube 1.** Phenylephrine was applied to a concentration of 10⁻⁴ M. After 5 min of incubation, 300 μl of supernatant KHS was pipetted out and deep frozen for storage.

**Tube 2.** A 5-min incubation with phenylephrine (10⁻⁴ M) was performed as with tube 1. Next, ACh was added in a cumulative fashion (10⁻⁷–10⁻⁵ M), similar to the myographical experiments.
Data Analysis and Statistical Procedures

Vessels were preincubated within the tube with rosiglitazone ($10^{-8}$ M, 20 min) as in the myographic experiments. An identical protocol as that carried out on tube 2 was then performed. The vascular tissue remaining in the tubes was dried out and weighed. The metabolites derived from thromboxane A$_2$ (TXA$_2$) and PG$_{12}$ [thromboxane B$_2$ (TXB$_2$) and 6-keto-PGF$_{1α}$, respectively] were determined in the supernatants using commercial immunoassay kits (Cayman Chemical, Ann Arbor, MI). Finally, data were normalized for dry weight.

Analytic Determinations in Blood Samples

Serum glucose, cholesterol, triglycerides, HDL-to-cholesterol ratios, urea, and creatinine were analyzed by standard methods. Plasma insulin was determined by enzyme immunoassay (Cayman Chemical).

Data Analysis and Statistical Procedures

$K^+$-KHS contractions were expressed as wall tension, i.e., wall force per unit length of the segment. This was fixed to 3.3 mm in the aorta and was variable for MRAs. In the case of phenylephrine dose-response curves, results were normalized by expressing them as a percentage of the force achieved by the $K^+$-KHS contraction. For ACh and SNP dose-response curves, results were expressed as a percentage of the force reached with phenylephrine ($10^{-6}$ M in MRAs or $10^{-5}$ M in aortae). Two parameters were calculated for every concentration-response curve: the maximum effect ($E_{max}$) that the substance being tested elicited and the concentration of this substance producing 50% of the $E_{max}$ ($pD_2$). All $E_{max}$ and $pD_2$ results are shown as means ± SE. Statistical significance was determined by a Student's unpaired $t$-test for two points except for the analysis of prostaglandin metabolites, for which a Student's paired $t$-test was used. The level of significance was set at $P < 0.05$.

Preparation of Drugs and Chemicals

Pioglitazone and rosiglitazone for in vitro experiments were purchased from Alexis Biochem (Lausen, Switzerland), and NS-398 and SQ-29,548 were from Cayman Chemical. Rosiglitazone for in vivo experiments was from GlaxoSmithKline (Glaxo Wellcome, Research Triangle Park, NC).

Table 3. ACh-induced relaxations of MRAs and aortae of WKY rats and SHROBs

<table>
<thead>
<tr>
<th>MRAs</th>
<th>WKY Rats</th>
<th>SHROBs</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{max}$, %</td>
<td>$pD_2$</td>
<td>n</td>
</tr>
<tr>
<td>Control</td>
<td>122 ± 2</td>
<td>5.97 ± 0.04</td>
</tr>
<tr>
<td>L-NAME</td>
<td>156 ± 10†</td>
<td>6.57 ± 0.08†</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>96 ± 10†</td>
<td>5.72 ± 0.13*</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Rosiglitazone (oral)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Aortae</td>
<td>68 ± 2</td>
<td>6.19 ± 0.07</td>
</tr>
<tr>
<td>L-NAME</td>
<td>173 ± 4†</td>
<td>7.11 ± 0.08*</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>32 ± 5†</td>
<td>6.36 ± 0.06</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Rosiglitazone (oral)</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$, number of segments. *$P < 0.05$ and †$P < 0.01$ compared with the control ACh curve of the same artery and strain; ‡$P < 0.01$ compared with the control ACh curve of WKY rats. Not every possible comparison was analyzed.
come, Aranda de Duero, Spain). All other compounds were obtained from Sigma Aldrich (Alcobendas, Spain). The glitazones NS-398 and SQ-29,548 were dissolved in DMSO (final concentration: 0.02%) to prepare working solutions. Indomethacin and TCP were prepared in ethanol freshly every experiment. Other compounds were prepared in distilled water. DMSO and ethanol were tested in preliminary experiments to rule out any vasoactive effects.

RESULTS

Physiological and Biochemical Parameters of WKY Rats and SHROBs

Table 1 shows various physiological and biochemical constants, all of which, except for creatinine, were significantly higher in SHROBs compared with healthy WKY rats. Effective lumen diameters of the MRAs from SHROBs were substantially smaller than those of healthy controls [WKY rats: 326 ± 5 μm (n = 88) and SHROBs: 265 ± 4 μm (n = 186), P < 0.01].

Vascular Contractility of Aortae and MRAs

The contractile responsiveness of mesenteric vessels to K⁺-KHS was higher in SHROBs (9.3 ± 0.2 mN/mm) compared with WKY rats (7.3 ± 0.2 mN/mm, P < 0.01), but that of quiescent aortic segments was similar between SHROBs (8.1 ± 0.2 mN/mm) and healthy WKY rats (8.2 ± 0.3 mN/mm).

MRAs from SHROBs challenged with phenylephrine elicited weaker contractions but were more sensitive to the adrenergic agonist than arteries from healthy controls (Fig. 1A and Table 2). Aortic segments, in turn, displayed the opposite contractile tendency in terms of E_max, which was superior in aortae from SHROBs but not in terms of
sensitivity to the adrenergic agonist, which was also higher in SHROBs (Table 2).

**Endothelium-Dependent and -Independent Relaxations**

MRAs and aortae from SHROBs displayed significantly lower relaxations to ACh compared with those of WKY rats. Sensitivity was also lower in the case of MRAs (Fig. 1B and Table 3). Endothelium-independent relaxations of the vascular smooth muscle assessed with SNP were severely impaired in SHROB MRAs compared with those of control rats (WKY rats: $E_{\text{max}} = 79 \pm 7\%$, $pD_2 = 7.16 \pm 0.25$, and $n = 9$; and SHROBs: $E_{\text{max}} = 36 \pm 3\%$, $pD_2 = 5.62 \pm 0.21$, and $n = 9$, both $P < 0.01$; Fig. 1C). Papaverine ($3 \times 10^{-4}$ M) fully relaxed microvessels from both strains of rats (Fig. 1C, inset).

**Role of Endothelium-Derived Substances in the Control of Vascular Contractility and Relaxation**

In the presence of L-NAME, responses to the adrenergic agonist were markedly enhanced in every vessel except for MRAs from SHROBs (Fig. 2 and Table 2). L-NAME increased the sensitivity to phenylephrine of all vessels (Table 2). Indomethacin diminished to different extents the relative force generated by the adrenergic agonist in every artery (Table 2).

Responses of MRAs to ACh under NO pathway blockade with L-NAME were unaffected in SHROBs and diminished, but not abolished, in the case of WKY rats, with a maximum relaxation of 60% and significantly lower $pD_2$ (Table 3 and Fig. 3, A and inset). In contrast to mesenteric microvessels, relaxations to ACh under NO inhibition were abolished in the aortae of both kinds of rats (Table 3 and Fig. 3, B and inset).

Indomethacin significantly potentiated ACh-induced responses of the aortae and MRAs of obese rats but not those of normotensive animals (Table 3 and Fig. 3). NS-398, but not SQ-29,548 or TCP, improved ACh-induced relaxations of MRAs from SHROBs (Table 3 and Fig. 4).

**Role of Superoxide Radicals in the Endothelium-Dependent and -Independent Relaxation of MRAs**

Endothelium-dependent relaxations to ACh were significantly enhanced in microvessels incubated with tempol.

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**Fig. 3. Effects of NO synthase or cyclooxygenase inhibition on ACh-induced responses of SHROB MRAs (A) and aortae (B). Insets: same in the corresponding WKY arteries. Values are means ± SE. *$P < 0.05$ and **$P < 0.01$ compared with the ACh control curve.**
In contrast, endothelium-independent relaxations to SNP were marginally and not significantly improved (Table 4).

**Acute Effects of Glitazones on Vascular Contractility**

The addition of either glitazone to MRAs diminished the average tension achieved when incubated with K\textsuperscript+\textsubscript{-H11001-KHS}, reaching significance (P < 0.05) in the case of rosiglitazone but not with pioglitazone (pioglitazone: 6.9 ± 1.3 mN/mm and rosiglitazone: 6.1 ± 0.9 mN/mm vs. control: 7.3 ± 0.2 mN/mm). Glitazones slightly increased the contractile responses to K\textsuperscript+\textsubscript{-stimulation of the aortae from obese rats (pioglitazone: 9.7 ± 0.4 mN/mm and rosiglitazone: 9.8 ± 0.5 mN/mm vs. control: 8.2 ± 0.3 mN/mm, P < 0.05). The presence of glitazones before adrenergic stimulation did not elicit any effect on phenylephrine contractions in any SHROB vessel (Table 2).

**Acute Effects of Glitazones on Endothelium-Dependent and -Independent Relaxations**

The presence of pioglitazone in the organ bath significantly increased the maximal relaxing ability of ACh in MRAs from obese rats, which occurred at an ACh concentration of 3 × 10\textsuperscript{-7} M (Table 3 and Fig. 5A). Rosiglitazone also increased the E\textsubscript{max} of ACh (Table 3) as well as the relaxations of most concentrations of the cholinergic agonist (Fig. 5A). In contrast, these drugs markedly diminished the sensitivity, but not the E\textsubscript{max}, of ACh of the aortae (Fig. 5B and Table 3).

Both kinds of SHROB arteries relaxed significantly more to SNP when glitazones were present, but the improvement was more patent in MRAs (Fig. 6 and Table 4).

**Chronic Effects of Rosiglitazone on Physiological and Biochemical Parameters**

Table 1 shows the physical and chemical features of obese rats exposed for 3 wk to rosiglitazone. Body weight was found to be significantly increased. Blood pressure tended to be lower without reaching significance. Insulin and serum lipids showed a marked reduction, whereas serum glucose, urea, and creatinine did not change. Heart rate was also found to be unchanged (318 ± 19 beats/min before treatment vs. 344 ± 9 beats/min after treatment, n = 4, P > 0.05). The effective lumen diameter

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### Table 4. Effects of glitazones on sodium nitroprusside-induced relaxations of MRAs and aortae of SHROBs

<table>
<thead>
<tr>
<th></th>
<th>E\textsubscript{max}, %</th>
<th>pD\textsubscript{2}</th>
<th>n</th>
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</thead>
<tbody>
<tr>
<td><strong>MRAs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>36 ± 3</td>
<td>5.62 ± 0.21</td>
<td>9</td>
</tr>
<tr>
<td>Tempol</td>
<td>42 ± 7</td>
<td>6.39 ± 0.26</td>
<td>4</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>49 ± 3*</td>
<td>6.12 ± 0.19</td>
<td>6</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td>48 ± 5*</td>
<td>6.15 ± 0.12*</td>
<td>8</td>
</tr>
<tr>
<td>Rosiglitazone (oral)</td>
<td>53 ± 3†</td>
<td>6.81 ± 0.21†</td>
<td>16</td>
</tr>
<tr>
<td><strong>Aortae</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>90 ± 1</td>
<td>7.35 ± 0.08</td>
<td>19</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>97 ± 1†</td>
<td>7.19 ± 0.13</td>
<td>10</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td>95 ± 2*</td>
<td>6.96 ± 0.25</td>
<td>9</td>
</tr>
<tr>
<td>Rosiglitazone (oral)</td>
<td>94 ± 1†</td>
<td>7.64 ± 0.07†</td>
<td>27</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of segments. *P < 0.05 and †P < 0.01 compared with the control sodium nitroprusside curve of the same artery. Not every possible comparison was analyzed.
of MRAs from rosiglitazone-treated rats was not different compared with untreated SHROBs (270 ± 14 μm, n = 23, P > 0.05).

**Chronic Effects of Rosiglitazone on Vascular Contractility**

Stimulation with K⁺-KHS in animals chronically treated with rosiglitazone did not cause different responses to those found in control untreated SHROB rats in either MRAs (9.3 ± 0.4 mN/mm) or the aorta (8.8 ± 0.4 mN/mm). In contrast, phenylephrine-induced responses were significantly attenuated in both kinds of arteries (Table 2).

**Chronic Effects of Rosiglitazone on Endothelium-Dependent and -Independent Relaxations**

Relaxing responses to ACh were discretely, albeit significantly deeper, in small arteries and aortae from animals chronically treated with rosiglitazone (Table 3 and Fig. 7).

Relaxations elicited by SNP were visibly improved in MRAs (Fig. 6A), both in terms of E_{max} and sensitivity (Table 4), and very slightly, but significantly, enhanced in aortae (Fig. 6B).

**Effects of Rosiglitazone on Prostaglandin Metabolite Release From SHROB Small Arteries and Aortic Segments**

On stimulation with ACh, the metabolite of TXA₂ (TXB₂) increased significantly in the supernatant of incubated MRAs from SHROB (n = 9, P < 0.05; Fig. 9A). The metabolite of PGI₂ (6-keto-PGF₁α) did not change significantly, although it showed a trend to increase followed by a recovery with rosiglitazone treatment (n = 8, P > 0.05; Fig. 9B). In contrast to SHROBs, ACh elicited no increase in MRAs from WKY rats, but there was a slight tendency to decrease the release of these prostaglandins (insets in Fig. 9, A and B). In aortae, ACh caused no change in prostaglandin release; however, rosiglitazone induced an increase in metabolite release, which was significant in the case of 6-keto-PGF₁α (Fig. 9, C and D).

**DISCUSSION**

The SHROBs used in the present study displayed the physical and biochemical traits of the rats originally described by Koletsky (7, 14), i.e., overweight, mild hypertension accompanied by a diminished luminal diameter in the resistance side of the circulation, exaggerated hyperinsulinemia, moderate hyperglycaemia, marked dyslipemia, and hyperuricaemia. Thus, these animals exhibit a close resemblance to the condition of metabolic syndrome. Because, to date, no systematic study on vascular endothelial and smooth muscle function has been performed in the SHROB, the most important objective of the
present work was to characterize the basic contractile and relaxant (endothelium-dependent and -independent) properties of SHROB vessels representative of microangiopathy (MRAs) and macroangiopathy (aortae).

**MRAs**

Dysfunction in the resistance circulation has been demonstrated in the condition of metabolic syndrome as a whole (27) as well as within the different individual pathological conditions that characterize this syndrome (3, 4, 30). Here, we showed that MRAs of SHROBs display less contractility to adrenergic stimulation, lower endothelium-dependent relaxation, and a loss in endothelium-independent relaxation (Fig. 1). Compared with WKY rats, phenylephrine-induced contractions of SHROB MRAs exhibited a lower control by NO, as demonstrated by the marked differences in the effects of L-NAME on the phenylephrine-induced contractions (Fig. 2, A and inset, and Table 2). Indomethacin lowered the force of the contractions in the MRAs of both strains, showing a comparable participation of prostaglandins (Fig. 2). It is plausible that the differences in the adrenergic responses between the two strains are related to a different pattern of α1- and α2-receptor distribution. This has not been studied in the SHROB, but in the case of SHRs, a diminished amount of α1-receptors has been reported (13).

Endothelium-dependent relaxations to ACh were unsurprisingly blunted in MRAs of rats with metabolic syndrome (Fig. 1B). The L-NAME experiments suggested a lack of NO release or bioavailability, as deduced by the inability of this drug to alter ACh-induced responses, especially when compared with WKY rats, which showed a significant fall in these responses under NO pathway blockade (Fig. 3, A and inset). A similar finding on NO bioavailability has been also reported in the Zucker fatty rat (8). Indomethacin improved ACh-induced relaxations in SHROB microvessels but not in WKY microvessels. These experiments suggest the existence of prostaglandin vasoconstrictors partially responsible for the impaired ACh-induced relaxations of the SHROB (Fig. 3, A and inset). Further analysis of the prostaglandin pathway in these microvessels revealed a higher production of cyclooxygenase 2-derived vasoconstrictor prostanoids, as suggested by the results obtained with the specific inhibitor NS-398. Individual prostaglandins, such as TXA2 or PGI2, could not be pinpointed pharmacologically using a TP receptor antagonist (SQ-29,548) or an inhibitor of PGI2 synthase (TCP) (Fig. 4). However, when quantified by enzyme immunoassay, an increase in the release of the TXA2 metabolite TXB2 was detected in SHROB MRAs but not in WKY MRAs, in which the basal release of TXB2 was patent lower compared with SHROBs (Fig. 9, A and inset). The PGI2 metabolite 6-keto-PGF1α showed a trend to augment in SHROB MRAs, whereas in WKY MRAs no such tendency was detected (Fig. 9, B and inset). The failure to pharmacologically detect an alteration in the production of a specific prostaglandin together with the immunoassay results suggest that a variety of different vasoconstrictor prostaglandins, not only the measured TXA2 and PGI2, are involved in the effects of ACh on SHROB arteries. An excess in TXA2 receptor-mediated vasoconstriction in microvessels has been linked to an overproduction of ROS leading to vascular dysfunction (9, 31). In the present experiments, superoxide scavenging with tempol significantly improved ACh-induced relaxations (Fig. 4), indicating that in MRAs of SHROBs, an excessive superoxide production (related or not with TXA2 receptor-mediated vasoconstriction) takes place as well.

Endothelium-independent relaxations, examined with SNP, were dramatically impaired in SHROB MRAs (Fig. 1C). This sort of impairment has not been reported before in other animal models of metabolic syndrome or hypertension. For instance, in the SHR, endothelium-independent relaxation is well known to be preserved (4). SNP elicits its vasodilatory effects by releasing exogenous NO independently of that generated by endothelial NO synthase. To test whether a vasodilator relaxes the smooth muscle through a different NO-independent mechanism (5), we tested papaverine. Interestingly, this vasodilator fully relaxed microvessels from both strains of rats (Fig. 1C, inset). Next, we hypothesized that the excess in oxygen radical production in the SHROB vessels, as indicated by previous experiments, reacts with exogenous NO. This possibility has also been suggested by some observations on the release of superoxide anions from endothelial cells and the inactivation of SOD, both when the vessel is treated with NO donors (22). Indeed, marked diminished endothelium-independent relaxations to SNP have been reported when vessels are devoid of endothelium (4). In our setup, coadministration of SNP and tempol failed to improve the relaxations, thus discarding that possibility. Therefore, the abnormal endothelium-independent relaxations of SHROBs are possibly due to a fault at the level of soluble guanylate cyclase. It has been reported in the stroke-prone SHR that MRAs display exaggerated responses to SNP (4). In contrast, others (29) have reported that obesity is characterized by impaired NO-independent vasodilation. Keeping these findings in mind, the results we obtained in

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**Fig. 8.** Effects of an oral 3-wk treatment with rosiglitazone on endothelium-independent relaxations to SNP of SHROB MRAs (A) and aortae (B). *P < 0.05 and **P < 0.01 compared with the control curve.
SHROBs are likely more related to the obesity rather than to the hypertensive condition.

Aortae

Aortic segments of metabolic syndrome rats exhibited a marked enhancement of phenylephrine-induced contraction. This cannot be ascribed to a deficient NO pathway, as with small arteries, since incubation with L-NAME visibly magnified the strength of the contractions. An inadequate release of prostaglandins cannot be assigned either to the excessive phenylephrine contractions, at least compared with WKY aortae, in which indomethacin elicited comparable effects to those of SHROBs (Fig. 2, B and inset). Still, the effects of L-NAME on phenylephrine-induced contractions were more noticeable in healthy animals than in SHROBs; thus, a possible downregulation of the NO pathway cannot be ruled out. Endothelium-dependent relaxations were, unlike those of MRAs, totally dependent on NO, both in healthy and metabolic syndrome aortae, as indicated by the abolition of the relaxations in L-NAME-incubated aortae. The aortic ACh-induced relaxations, in contrast to those of MRAs, were relatively well preserved compared with those of WKY rats (Fig. 3, B and inset). This is possibly because neither TXA₂ nor PGI₂ increased upon ACh treatment (Fig. 9, C and D). Application of indomethacin significantly improved the relaxations of SHROB aortae and, at the same time, abolished prostaglandin metabolite release (not shown). Thus, a decrease in these vasoconstrictor prostaglandins is probably what is expected on ACh stimulation in healthy aortae. Indeed, indomethacin did not further improve ACh-induced responses in WKY rats (Fig. 3B, inset).

**Glitazone Treatment**

Rosiglitazone, which was orally provided to SHROBs for 3 wk, elicited salutary effects on the rats’ insulin sensitivity as well as on serum lipid concentrations. In addition to these effects, there was an increase in body weight. All these actions have never been reported for a glitazone in the SHROB and are the ones expected for glitazone therapy (11, 19). These observations reinforce the adequacy of the SHROB as a model for metabolic syndrome research suitable for pharmacological studies. Thus, we sought to determine the effects of glitazones on vascular function of the aorta and MRAs in these rats.

Incubation of the vessels with either pioglitazone or rosiglitazone produced trivial effects on phenylephrine-induced contractions. However, the effects on ACh-induced relaxations were totally opposite in large and small arteries. Whereas in microvessels the glitazones improved endothelium-dependent relaxations, in aortae the presence of these drugs diminished the sensitivity to the cholinergic agonist (Fig. 5). In this regard, SHROB aortae behaved very similarly to SHR aortae (16). In that work, we reported that glitazones do not improve endothelial function of SHR aortae per se but rather ameliorated endothelial dysfunction only when cyclooxygenase was inhibited (16). On the other hand, endothelium-independent relaxation was improved by glitazone incubation of small vessels whose relaxant properties were already reduced (Fig. 6). In aortae, which already displayed a nearly 100% relaxation, glitazones had a very marginal, yet significant, effect.
In an attempt to find a prostaglandin-dependent mechanism of endothelial function changes caused by glitazones, we measured the vasorestrictor prostaglandins TXA₂ and PGI₂, as released from microvessels and aortae incubated with ACh and rosiglitazone. In the case of MRAs from SHROBs, prostaglandin metabolites, which had been increased by ACh, did not change or tended to diminish in the presence of rosiglitazone (Fig. 9, A and B). In sharp contrast to small arteries, in the aorta both metabolites, which were unchanged by ACh, increased when rosiglitazone was present (although the TXB₂ results did not reach significance, the trend was to rise; Fig. 9, C and D). When these analytic findings are compared with the pharmacological ones, it is tempting to suggest that, in MRAs, endothelial function is impaired partly because of an increased release of prostanoids and this can be alleviated with rosiglitazone and improved with cyclooxygenase inhibition (Figs. 3A and 4). In contrast, in the aorta, endothelial function is comparatively preserved and rosiglitazone does not improve it (ACh sensitivity is actually diminished; Fig. 5B). These observations are in keeping with the analytic prostaglandin metabolite findings (Fig. 9, C and D).

Further reassurance of the beneficial vascular effects of glitazones in long-term therapeutic conditions is provided by the results obtained from SHROBs orally treated with rosiglitazone. These experiments showed that MRAs and aortae displayed a diminished contractility to adrenergic stimulation and an improved endothelium-dependent as well as endothelium-independent relaxation (Figs. 7 and 8). Oral rosiglitazone did not exactly reproduce the acute experiments in all conditions, such as ACh in the aorta. The reasons for this are unknown, but it is worth noting that, while acute administration of glitazones is likely to be peroxisome proliferator-activated receptor (PPAR-γ) independent because there is not enough time for protein synthesis, the oral treatment protocol involves PPAR-γ-dependent actions (19). The necessary involvement of differential PPAR-γ-dependent and -independent pathways inevitably confounds the final results.

Conclusions

In summary, we explored, for the first time, the vascular reactivity and effects of glitazones in a rat model of metabolic syndrome, the SHROB, at a macrovascular and microvascular level. We report that ACh- and SNP-induced relaxations are impaired to different extents in this model and that glitazones appear to have beneficial vascular effects, especially in MRAs.

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