Kidney International, Vol. 68 (2005), pp. 2787–2793

Role of AT-1 receptor in regulation of vascular MCP-1, IL-6, PAI-1, MAP kinase, and matrix expressions in obesity

NOSRATOLA D. VAZIRI, ZHONG-GAO XU, ASHKAN SHAHKARAMI, KEVIN T. HUANG, BERNARDO RODRÍGUEZ-ITURBE, and R. NATARAJAN

Division of Nephrology and Hypertension, University of California at Irvine, Irvine, California; Gonda Diabetes Research Center, Beckman Research Institute of the City of Hope, Duarte, California; and Hospital Universitario, Universidad del Zulia, Maracaibo, Venezuela

Role of AT-1 receptor in regulation of vascular MCP-1, IL-6, PAI-1, MAP kinase, and matrix expressions in obesity.

Background. Metabolic syndrome has emerged as the major cause of atherosclerosis. The associated atherosclerosis is accompanied by and, in part, due to inflammation. In an attempt to explore the molecular sources of vascular inflammation and possible involvement of renin-angiotensin system, we studied obese Zucker rats, which exhibit all features of metabolic syndrome.

Methods. Seven-week-old male obese Zucker rats were randomized to losartan-treated (100 mg/L drinking H_2O) and untreated groups. Lean Zucker rats served as controls. After four months, aortas were obtained and processed for various determinations by reverse transcription-polymerase chain reaction (RT-PCR) and Western blot and immunohistochemical analysis for collagen type IV.

Results. Compared to the lean controls, obese Zucker rats showed significant increases in collagen staining, as well as expressions of collagen, fibronectin, plasminogen activator inhibitor-1, and two major proinflammatory mediators (i.e., interleukin-6 and monocyte chemoattractant protein-1). This was associated with significant increases in p38 and ERK1/2 mitogen activated protein kinase activities, as well as marked up-regulation of angiotensin II type 1 receptor (AT-1R) mRNA expression. These abnormalities were prevented by administration of the AT-1R blocker (ARB).

Conclusion. The untreated obese Zucker rats exhibit increased matrix protein accumulation in the aorta and marked up-regulations of proinflammatory and profibrotic pathways. These abnormalities are associated with up-regulation of AT-1R and are prevented by AT-1R blockade pointing to the potential role of AT-1R activation.

Atherosclerosis is the primary cause of coronary disease, stroke, ischemic nephropathy, and peripheral vascular disease and, as such, represents the main cause of morbidity and mortality in the industrialized societies. Metabolic syndrome, otherwise known as syndrome X or insulin resistance syndrome is the principal cause of diabetes and atherosclerosis in the developed countries [1, 2]. The syndrome is defined as an aggregation of several proatherogenic conditions, including insulin resistance, hyperinsulinemia, and some combination of dyslipidemia, obesity, endothelial dysfunction, hypercoagulability, and hypertension [3–7]. In addition, inflammation is frequently present and plays a major role in atherogenesis and cardiovascular disease in metabolic syndrome [8–10].

Increasing evidence suggests that insulin resistance causes inflammation and inflammation promotes insulin resistance [11–13]. Given the central role of inflammation in the pathogenesis of atherosclerosis, the associated inflammation must be involved in the atherogenic diathesis in the metabolic syndrome. Inflammatory chemokines, particularly monocyte chemoattractant protein-1 (MCP-1), and cytokines participate in macrophage recruitment, whereas metaloproteinase inhibitors, particularly plasminogen activator inhibitor-1 (PAI-1) and activated mitogen activated protein kinases (MAPK), promote matrix accumulation, cell migration, and proliferation, events that are critical in atherogenesis. Production of several cytokines, including tumor necrosis factor-alpha and interleukin-6 (IL-6) is increased in obesity and insulin resistance [11].

It is of note that (renin)-angiotensin system (RAS) activity is augmented in the metabolic syndrome [14], and blockade of the RAS ameliorates insulin resistance and prevents/delays onset of type 2 diabetes [15–20]. Moreover, activation of angiotensin II type 1 receptor (AT1R) by angiotensin II promotes inflammation, oxidative stress, and cardiovascular remodeling [21, 22]. Thus, AT-1 receptor activation may be involved in inflammation and atherogenesis in metabolic syndrome.

Metabolic syndrome is frequently caused by environmental conditions, notably sedentary lifestyle, excess caloric intake, and high-fat, high-refined sugar diet, as

Key words: obesity, angiotensin II type 1 receptor, atherosclerosis, inflammation, syndrome X, insulin resistance, metabolic syndrome.

Received for publication June 21, 2005
and in revised form July 11, 2005
Accepted for publication July 20, 2005

well as a variety of acquired and hereditary disorders. Autosomal-recessive mutation of gene, encoding leptin receptor in the obese Zucker rat results in hyperphagia and metabolic syndrome, which manifests as obesity, insulin resistance, hyperinsulinemia, impaired glucose tolerance, and hyperlipidemia [23–26]. Obese Zucker rats are commonly used as a model to study metabolic syndrome.

In view of the association of metabolic syndrome with increased RAS activity, inflammation, and vascular disease, we hypothesized that the pathway involved in inflammation and remodeling may be activated in the aorta of the obese Zucker rats. We further considered that long-term blockade of RAS may reverse dysregulation of these pathways. Accordingly, we measured AT1R, MCP-1, IL-6, PAI-1, matrix proteins (collagen and fibronectin), as well as activities of p38 and ERK1/2 MAPK in the aortas of the obese and lean Zucker rats. We further examined the effects of AT1R blockade on the measured parameters.

METHODS

Materials

Antibodies for phosphospecific and nonphospho-p38 and -ERK1/2 MAPK were from Cell Signaling (Beverly, MA, USA); collagen α (IV) antibody was from Santa Cruz Biotechnology (Santa Cruz, CA, USA); Supersignal chemiluminescence reagent from Pierce (Rockford, IL, USA); Relative multiplex reverse transcription-polymerase chain reaction (RT-PCR) kits and primers for Quantum RNA 18S internal standards were from Ambion, Inc. (Austin, TX, USA); RNA-STAT60 reagent was from Tel-Test (Friendswood, TX, USA). Losartan (ARB) was obtained from Merck Co. (Westpoint, PA, USA).

Animals and experimental design

All animal studies were conducted under a protocol approved by the Animal Care and Use Committee of the University of California, Irvine. Seven-week-old male lean Zucker rats ($N = 6$) and male obese Zucker rats ($N = 12$) were used in this study. Obese animals were further randomized into two groups of six rats each. One group of obese Zucker rats was administered losartan in the drinking water (100 mg/L) for four months. Under general anesthesia, the rats were sacrificed by exsanguinations using cardiac puncture. Aortas were removed and stored at −70°C for further study. Additional specimens were fixed in 10% formalin for histologic evaluation. Tail arterial pressure, serum glucose, cholesterol, and triglyceride concentrations were determined by standard methods.

Table 1. Primer sequences for amplification of various transcripts

Target	Sequence	Product *bp*
MCP-1		191
Sense	5′-CCTGCTGCTACTCATTCAC-3′	
Antisense	5′-TCTCACTTGGTTCTGGTCC-3′	
IL-6		256
Sense	5′-GGATACCACCCACAACAGAC-3′	
Antisense	5′-GAAACGGAACTCCAGAAGAC-3′	
Collagen α1 (I)		413
Sense	5′-GTG AAC CCG GCA AAC AAG GT -3′	
Antisense	5′-CTG GAG ACC AGA GAA GCC AC -3′	
Fibronectin		446
Sense	5′-GCAAGCCTGAACCTGAAGAGACC-3′	
Antisense	5′-CCTGGTGTCCTGATCATTGCATC-3′	
PAI-1		339
Sense	5′-GCCTCCAAAGACCGAAAT-3′	
Antisense	5′-GTCGTTGATGATGAATCTGGCTC-3′	

Relative and competitive RT-PCR

Total RNA was isolated using RNA-STAT60 reagent according to the manufacturer's instructions. cDNA was synthesized with 1 μg of RNA using murine leukemia virus reverse transcriptase and random hexamers. Primers for the 18S ribosomal RNA (489-bp or 324-bp) were included in each reaction as an internal control. The primers used are summarized in Table 1. Relative reverse transcription-polymerase chain reactions (RT-PCRs) were performed as described previously [25].

For AT1R mRNA expression, we used a quantitative competitive RT-PCR method as described previously [26]. The AT1R competitor cDNA (212 bp) used as internal standard was designed to contain the same base pair sequence as the target cDNA that would allow efficient priming, but had a portion deleted so that the competitor PCR-generated fragment could be easily distinguished electrophoretically by size. Band densities were analyzed by laser densitometry (Bio-Rad Laboratories, Hercules, CA, USA). The RT-PCR products were separated by electrophoresis and competitive PCR measurements were expressed as a ratio of the wild-type divided by the mutant band densities as described previously [27].

Immunohistochemical studies

Aorta tissues for immunohistochemical staining were fixed in 10% neutral buffered formalin and paraffin embedded by standard techniques, and 5 micron sections were utilized. Collagen α (IV) staining was performed using a commercial kit (Dako Corporation, Carpinteria, CA, USA) according to the manufacturer's instructions and as described previously [28].

Western blot analysis

Tissue samples were lysed in sodium dodecyl sulfate (SDS) sample buffer [2% SDS, 10 mmol/L Tris-HCl,