Available online at www.sciencedirect.com
SCIENCE DIRECT
PEPTIDES
Peptides 26 (2005) 1323–1330
www.elsevier.com/locate/peptides

Increases of spinal kinin receptor binding sites in two rat models of insulin resistance

Adil El Midaoui [a,1], Brice Ongali [a,1], Mihai Petcu [a], Donata Rodi [a], Jacques de Champlain [a], Witold Neugebauer [b], Réjean Couture [a,*]

[a] *Department of Physiology, Faculty of Medicine, Université de Montréal, C.P. 6128, Succ. Centre-ville, Montréal, Que., Canada H3C 3J7*
[b] *Department of Pharmacology, Faculty of Medicine, Université de Sherbrooke, Sherbrooke, Que., Canada J1H 5N4*

Available online 12 April 2005

Abstract

An autoradiographic study was conducted to determine whether kinin receptors are altered in the rat spinal cord in two experimental models of chronic hyperglycemia and insulin resistance. Sprague-Dawley rats were given 10% D-glucose in their drinking water alone or with insulin (9 mU/kg/min with osmotic pumps) for 4 weeks. Both groups and control rats were treated either with a normal chow diet or with an alpha-lipoic acid-supplemented diet as antioxidant therapy. After 4 weeks of treatment, glycemia, insulinemia, blood pressure, insulin resistance index, the production of superoxide anion in the aorta and the density of B_1 receptor binding sites in the dorsal horn were significantly increased in the two models. These effects were prevented or attenuated by alpha-lipoic acid. In contrast, B_2 receptor binding sites of most spinal cord laminae were increased in the glucose group only and were not affected by alpha-lipoic acid. Results show that chronic hyperglycemia associated with insulin resistance increases B_1 and B_2 receptor binding sites in the rat spinal cord through distinct mechanisms, including the oxidative stress for the B_1 receptor.

Keywords: Bradykinin; B_1 receptor; B_2 receptor; Spinal cord; Diabetes mellitus; Hyperglycemia; Insulin resistance; Hypertension; Oxidative stress

1. Introduction

Compelling evidence suggests a role for kinins as mediators of central neuronal pathways associated with the transmission of nociceptive information and autonomic control of arterial blood pressure [8,9]. These peptides signal through the activation of two transmembrane G-protein-coupled receptors denoted as B_1 and B_2 [31]. The widely distributed B_2 receptor is constitutive and mediates the majority of the biological effects of kinins [8,9], while the B_1 receptor is either absent or underexpressed in healthy animals. The latter receptor is induced and up-regulated following tissue injury, by bacterial endotoxins or in the presence of cytokines such as interleukin-1β and the tumour necrosis factor alpha [11,21]. The induction of B_1 receptors involves the transcriptional nuclear factor κB (NF-κB) and the mitogen-activated protein kinase (MAP-kinase) [23,28,33]. Since NF-κB is activated by hyperglycemia and the oxidative stress [10,38], B_1 receptor is thought to be involved in the development of diabetic complications. Rodents treated with streptozotocin (STZ), an acute model of type 1 diabetes, exhibited an up-regulation of B_1 receptors in several tissues where they mediate several biological functions, including paw edema and increased vascular permeability [3,34], mononuclear and polymorphonuclear leukocyte migration [36], vasodilation of retinal microvessels [1], inhibition of hyperglycemia-induced MAP-kinase activation in renal glomeruli [20], spinal autonomic control of cardiovascular function [5], and thermal hyperalgesia [9,15]. Recent molecular evidence confirmed the up-regulation of B_1 receptors in the thoracic spinal cord of streptozotocin-diabetic rats under acute hyperglycemia (2–21 days) [26]. However, the deficit in insulin as contributing factor and the

* Corresponding author at: Université de Montréal, Pavillon Paul-G.-Desmarais, 2960, Chemin de la Tour, Montréal, Que., Canada H3T 1J4. Tel.: +1 514 343 7060; fax: +1 514 343 2111.
E-mail address: rejean.couture@umontreal.ca (R. Couture).
[1] Both authors contributed equally to this work.

0196-9781/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.peptides.2005.03.028

possibility that chronic hyperglycemia can also up-regulate B_1 receptors remain unknown. It is also uncertain whether the oxidative stress that occurs during chronic hyperglycemia may affect the expression of B_1 and/or B_2 receptors. Thus, the aim of the present study was to examine whether B_1 and B_2 receptor binding sites are increased in the rat spinal cord in two experimental models of diabetes with insulin resistance. Because alpha-lipoic acid prevented the development of arterial hypertension, the oxidative stress and attenuated the insulin resistance in rats fed with glucose for 3–4 weeks [12,13], the second objective was to evaluate the effect of a diet supplemented with this antioxidant on the densities of B_1 and B_2 receptor binding sites in the rat spinal cord. Effects of the various treatments were also assessed on systemic blood pressure, plasma levels of glucose and insulin, insulin resistance and the basal production of superoxide anion in the aorta.

2. Materials and methods

2.1. Animals and protocols

Male Sprague-Dawley rats weighing 230–260 g were purchased from Charles River, St-Constant, Que., Canada. They were housed under a 12 h light-dark cycle in a room with controlled temperature (23 °C) and humidity (50%) with food and tap water available ad libitum. Two models of insulin resistance were used as described earlier [12–14].

First model, rats received 10% D-glucose in their drinking water for 4 weeks. Second model, rats received 10% D-glucose in their drinking water for 4 weeks along with an infusion of pork insulin (9 mU/kg/min, Sigma) using an Alzet 2002 osmotic pumps (Alza Corporation, Palo Alto) during the last 2 weeks. Both groups and control rats were treated with a normal chow diet (Charles River Rodent) or with a diet containing alpha-lipoic acid (1000 mg/kg feed) (Ren's Feed Supplies Limited). Systolic blood pressure was measured by tail-cuff photoplethysmography (Harvard Apparatus Ltd.) and calculated as the average of five determinations at the end of the treatment. Overnight fasted rats were sacrificed by decapitation under anesthesia with CO_2 inhalation. The blood, the thoracic aorta and spinal cord were removed for the measurements of glucose and insulin, superoxide anion and kinin receptor binding, respectively. The care of animals and research protocols were in compliance with the guiding principles for animal experimentation as enunciated by the Canadian Council on Animal Care and approved by the Animal Care Committee of our University.

2.2. Laboratory analysis

Blood was rapidly collected from sectioned carotids and immediately transferred into a chilled tube containing 0.63 mg/ml heparin. The plasma was separated from blood cells by centrifugation and kept frozen at −20 °C for the later measurement of glucose and insulin. Plasma glucose concentrations were measured with a glucometer (Elite, Bayer Inc., Toronto, Canada) and plasma insulin levels were determined by radioimmunoassay (Rat insulin RIA kit, Linco Research, St. Charles, MO) using 100 μl of plasma. The Homeostasis Model Assessment (HOMA) was used as an index of insulin resistance and calculated with the following formula: [insulin (μU/ml) × glucose (mM)/22.5] [29].

2.3. Superoxide anion measurement

Superoxide anion production was measured in isolated aortic rings using the lucigenin-enhanced chemiluminescence method as described previously [22]. Briefly, 5 mm aortic rings were pre-incubated in Krebs-Hepes buffer (saturated with 95% O_2 and 5% CO_2, at room temperature during 30 min) and then transferred to a glass scintillation vial containing 5 μmol/L lucigenin for the determination of basal O_2-levels. The chemiluminescence was recorded every minute for 15 min at room temperature by a liquid scintillation counter (Wallac 1409, Tuku, Finland). Background counts were determined from vessel-free incubation media and substracted from the readings obtained with vessels. Lucigenin counts were expressed as cpm/mg of dry weight of tissue.

2.4. Peptide iodination

Iodination of HPP-desArg^{10}-Hoe 140 and HPP-Hoe 140 was performed according to the chloramine T method [17]. Briefly, 5 μg of peptide were incubated in 0.05 M phosphate buffer for 30 s in the presence of 0.5 mCi (18.5 MBq) of ^{125}INa and 220 nmol of chloramine T in a total volume of 85 μl. The monoiodinated peptide was then immediately purified by high pressure liquid chromatography on a C4 Vydac column (0.4 mm × 250 mm) (The Separations Group, Hesperia, CA) with 0.1% trifluoroacetic acid and acetonitrile as mobile phases. The specific activity of the iodinated peptides corresponds to 2000 cpm/fmol or 1212 Ci/mmol.

2.5. Tissue preparation for autoradiography

After decapitation, spinal cord segments (T8–T11) were removed and immediately frozen in 2-methyl butane cooled at −45 to −55 °C with liquid nitrogen, and then stored at −80 °C until use. Matched spinal cord segments (T9–T10) of the same group of rats ($n = 4$) were mounted together (four spinal cords per gelatine block) and serially cut into 20 μm thick coronal sections on a cryostat (−11 to −13 °C). A total of six slices per slide were alternatively thaw-mounted on 0.2% gelatine/0.033% chromium potassium sulfate coated slides. Three slides were taken for the total binding and two slides (adjacent sections) for the non-specific binding. A total of 10 slides (240 sections) were obtained for each group studied and kept at −80 °C until use.