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Differential effect of losartan in female and male spontaneously hypertensive rats

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Abstract

We demonstrated that the decreased response to acetylcholine observed in aorta of male and female spontaneously hypertensive rats is corrected after sustained (15 days) reduction of blood pressure levels by losartan. In order to verify if the same occurs in resistance vessels, vascular diameter changes induced by topical application of acetylcholine and bradykinin (endothelium-dependent vasodilators) and sodium nitroprusside (endothelium-independent vasodilator) to mesenteric arterioles studied in vivo, in situ were determined in rats treated with losartan for 24 h (acute) or 15 days (chronic). Rats that presented similar reduction (in %) of the blood pressure levels after losartan treatment were chosen. Sodium nitroprusside induced similar responses in losartan-treated and untreated male or female SHR. Whereas in female SHR, losartan corrected the diminished arteriolar response to endothelium-dependent vasodilators after acute and chronic treatment, in male SHR this correction only occurred after chronic treatment. Thus, losartan corrected the endothelial dysfunction more easily in female than in male SHR and independently of the normalization or the magnitude of the reduction of the blood pressure levels. In an attempt to explain the difference, we evaluated the losartan effect on nitric-oxide synthase (NOS) activity and angiotensin II AT1 and AT2 receptor gene expression in these animals. In male and female SHR, NOS activity and AT1 receptor expression were not altered by acute or chronic treatment. On the other hand, AT2 receptor expression was augmented only in female SHR by these treatments. Therefore, augmented AT2 receptor expression, but not alteration of NOS activity or AT1 receptor expression, might explain the difference observed.

Keywords: Vascular reactivity; SHR; Losartan; Angiotensin AT2 receptor; Nitric-oxide synthase; Angiotensin AT1 receptor

Introduction

Correction of the endothelial dysfunction seems to be gender related. In a study from our laboratory utilizing male and female SHR treated with enalapril we demonstrated that, though females are more sensitive than males to the antihypertensive effect of this drug, the diminished microvascular response to acetylcholine was corrected only in males (Nigro et al., 1997). Similarly, although female SHRs were more responsive than males to the antihypertensive effect of losartan, the decreased response to acetylcholine in aorta was corrected in both male and female SHR only after chronic treatment with this agent (Silva-Antonialli et al., 2000). If the same occurs in resistance vessels remained to be determined.

Although the mechanisms underlying losartan effect on vascular reactivity of male and female SHRs are not completely understood, a recent work from our laboratory demonstrated an important effect of this antihypertensive agent, administered chronically, reducing the overproduction of superoxide anion (O_2^-) and, in consequence, augmenting NO bioavailability in male and female SHRs. Male SHR exhibited a higher (65%) and females a lower (31%) attenuation of O_2^- generation (Dantas et al., 2004). These data suggest that other mechanisms might contribute to the effect of losartan on the vascular reactivity, mainly in female SHR.

Thus, the aim of the present study is to verify if the correction of vascular responses to endothelium-dependent

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vasodilators after losartan treatment, observed in aorta, occurs in resistance vessels, at a dose that induces similar reduction in blood pressure levels. The influence of the treatment on nitric-oxide synthase (NOS) activity, angiotensin II AT1 and AT2 receptor expression will also be investigated in male and female SHR.

Methods

All procedures used in this study were approved and performed in accordance with guidelines of the Ethics Committee of the Institute of Biomedical Science, University of São Paulo (protocol no 156/2001).

Animals

Male and female SHRs (from breeding colony at the Institute of Biomedical Science, University of São Paulo, Brazil) at the age of 14–16 weeks and weighing 200–300 and 130–190 g (at the moment of the experiments), respectively, were used. Untreated female and male Wistar, age- and weight-matched, rats were used for comparison in the microvascular reactivity study. All rats had free access to food and water and were maintained in a room at 22 °C with a 12 h light cycle and 60% humidity. SHRs were divided into six groups: 1) untreated male; 2) untreated female; 3) male treated with losartan for 24 h (acutely); 4) female treated with losartan for 24 h; 5) male treated with losartan for 15 days (chronically); 6) female treated with losartan for 15 days. In all of the treated groups, losartan was suspended in tap water and administered daily at dose of 15 mg/kg by gavage. To exclude the interference of differences in magnitude of the effect of losartan on blood pressures after treatment we chose the animals that presented similar reduction in blood pressure levels (around 13%) with the losartan dose employed. Diminished reactivity to angiotensin II in the mesenteric arterioles, in vivo, and reduction on blood pressure levels were used as criteria for the effectiveness of the acute and chronic treatment.

Measurement of arterial blood pressure

Arterial blood pressure was determined in conscious SHR and Wistar rats by an indirect tail–cuff method, using a programmed E and M Instrument Co. electrosphygmomanometer (Narco Bio System, TX, USA), always in the same period of the day (morning). Rats were preheated at 40 °C for 10 min, and then three stable consecutive measurements of blood pressure were averaged, after a 4-day training period, in which the animals were placed in the contention cylinder for a few minutes, in an attempt to reduce the stress that might interfere in the results. Values were obtained before and after treatment of the animals with losartan. The cuff pressure was controlled automatically and the systolic pulses detected by the pulse transducer were monitored with the audio signal. The cuff size was regulated in accordance with each animal.

Oestrus induction

To induce oestrus, female SHR and Wistar rats received 17-β estradiol (150 μg/rat in mineral oil, s.c.) 24 h before testing. Oestrus was determined by microscopic examination of vaginal smears obtained with a cotton swab, immediately before the rat was used. The plasma concentrations of 17-β estradiol and progesterone were measured by radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, USA) to assess if its levels were similar among the groups. The oestrus phase was established to avoid the influence of the cyclic estrogen variation in our study, which could alter the results.

Procedures with mesenteric microvessels in situ

The SHR and Wistar rats were anesthetized with chloral hydrate, dissolved in saline (500 mg/kg, s.c.) and the mesentery was arranged for microscopic observation in situ, according to Zweifach (1948) with slight modifications (Fortes et al., 1983). In brief, the animals were kept on a special board, heated at 37 °C, which include a transparent plate where the tissue to be transilluminated is placed. The mesentery was kept moist and warm by irrigating the tissue with 37 °C Ringer Locke's solution, pH 7.2–7.4, containing 1% gelatin. The composition of the solution was (mM): 154.0 NaCl, 5.6 KCl, 2.0 $CaCl_2{\cdot}2H_2O$, 6.0 $NaHCO_3$ and 5.5 glucose. A 500-line television camera (JVC, Tokyo, Japan) was combined with a tri-ocular microscope to facilitate observation of the enlarged image (3400×) on the video screen. An image-splitting micrometer was adjusted to the phototube of the microscope (Carl Zeiss, Jena, Germany), shearing the optical image into two separate images, one displaced with respect to the other. By rotating the image splitter in the phototube, the shearing was maintained at right angles to the long axis of the vessel. The displacement of one image from the other allowed measurement of the vessel diameter (Baez, 1966).

Blood vessels were classified according to their branching order beginning at the capillary level and reaching up to the arteriolar side (Gore and Bohlen, 1977). The smallest precapillary arterioles were classified as A_4, fed by the terminal arterioles (A_3) branching from large arterioles (A_2). A_2 arterioles (15–25 μm) were selected for this study and any changes in vessel diameter were estimated following the topical application of acetylcholine (24 nmol), bradykinin (42 pmol), sodium nitroprusside (58 nmol) and angiotensin II (0.14 pmol). The doses of vasodilator agents were selected based on previous works from our laboratory and were the lowest doses that caused maximum vasodilatation (Fortes et al., 1989). The angiotensin II dose was chosen because of promoted expressive vasoconstriction in untreated SHR which was effectively antagonized by losartan treatment both in male and female. The drugs, dissolved in Ringer Locke's solution, were added to the preparation in a standard volume of 15 μL and were removed by washing with warmed Ringer Locke's solution. A given section of the vascular bed was tested only once and no more than two drugs were used on a single rat.