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Des-aspartate angiotensin I (DAA-I) reduces endothelial dysfunction in the aorta of the spontaneously hypertensive rat through inhibition of angiotensin II-induced oxidative stress

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ABSTRACT

Des-aspartate angiotensin I (DAA-I), an endogenous nonapeptide, counteracts several effects of angiotensin II on vascular tone. The aim of this study was to investigate the acute protective effect of DAA-I on endothelial function in the spontaneously hypertensive rat (SHR) as well as its effect on angiotensin II-induced contractions and oxidative stress. Aortic rings were incubated with DAA-I (0.1 μM) for 30 min prior to the assessment of angiotensin II-induced contractions (0.1 nM–10 μM) in WKY and SHR aortas. Total nitrate and nitrite levels were assessed using a colorimetric method and reactive oxygen species (ROS) were measured by dihydroethidium (DHE) fluorescence and lucigenin-enhanced chemiluminescence. The effect of DAA-I was also assessed against endothelium-dependent and -independent relaxations to acetylcholine and sodium nitroprusside, respectively. Angiotensin II-induced contractions were significantly reduced by DAA-I, losartan and tempol. Incubation with ODQ (soluble guanylyl cyclase inhibitor) and removal of the endothelium prevented the reduction of angiotensin II-induced contractions by DAA-I. Total nitrate and nitrite levels were increased in DAA-I, losartan and tempol treated-SHR tissues while ROS level was reduced by DAA-I and the latter inhibitors. In addition, DAA-I significantly improved the impaired acetylcholine-induced relaxation in SHR aortas whilst sodium nitroprusside-induced endothelium-independent relaxation remained unaffected. The present findings indicate that improvement of endothelial function by DAA-I in the SHR aorta is mediated through endothelium-dependent release of nitric oxide and inhibition of angiotensin II-induced oxidative stress.

1. Introduction

Hypertension is a multifactorial disease that involves complex interactions between homeostatic control mechanisms and environmental factors [1]. The abnormal vascular tone and blood vessel wall remodeling characteristic of hypertension are major risk factors for vascular disease [1,2]. Chronic elevation of arterial blood pressure is associated with endothelial dysfunction [3]. The hallmark of endothelial dysfunction is impaired endothelium-dependent vasodilatation due to attenuated production and release of nitric oxide (NO) and other endothelium-derived relaxing factors (EDRFs) and/or increased production of endothelium-derived contracting factors (EDCFs) [4,5]. The abnormal vascular reactivity in blood vessels of SHR is due in part to overproduction of reactive oxygen species (ROS), in particular superoxide anions which scavenge NO and reduce its bioavailability and thus contribute to the development of endothelial dysfunction [6–10].

The activity of the renin–angiotensin system (RAS) is enhanced in the SHR which leads to a decreased NO bioavailability [11]. RAS contributes to the regulation of arterial blood pressure in health and disease [12,13]. It does so primarily due to the vasoactive properties of angiotensin II and the sodium retaining properties of aldosterone. In particular, angiotensin II stimulates a number of cellular signaling pathways by binding to two distinct subtypes of specific G-protein coupled angiotensin receptors, AT_1R and AT_2R [14]. AT_1R coupling to G-proteins activates phospholipase C to form second messengers including inositol triphosphate (IP_3) and diacylglycerol (DAG) which increases the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) and eventually vascular tone [14]. AT_1R activation also stimulates nicotinamide adenine dinucleotide

Abbreviations: ACE, angiotensin converting enzyme; AT_1R, angiotensin II type 1 receptor; AT_2R, angiotensin II type 2 receptor; cGMP, cyclic guanosine monophosphate; DAA-I, Des- aspartate angiotensin I; DAG, diacylglycerol; EDCF, endothelium-derived contracting factor; EDRF, endothelium-derived relaxing factor; eNOS, endothelial nitric oxide synthase; IP_3, inositol triphosphate; L-NAME, N^G-nitro-L-arginine methyl ester; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; ODQ, 1*H*-[1,2,4]-oxadiazolo [3,4-a]quinoxalin-1-one; DHE, dihydroethidium; RAS, renin-angiotensin system; ROS, reactive oxygen species; SHR, spontaneously hypertensive rat; WKY, Wistar-Kyoto rat.

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phosphate (NAD(P)H) oxidase and enhances the production of reactive oxygen species (ROS) which leads to vascular constriction, remodeling and inflammation resulting from the expression of adhesion molecules and the release of cytokines and chemokines [15,16]. AT_2R activation has the opposite functional effects as it enhances bradykinin production which in turn stimulates NO release and promotes vasodilatation [17,18].

Des-aspartate angiotensin I (DAA-I) is an endogenous nonapeptide formed by an alternative degradation pathway of angiotensin I to angiotensin III bypassing the formation of angiotensin II [19,20]. DAA-I attenuates the central pressor action of angiotensin II and III in the SHR [21]. It also exerts cardioprotective effects in pathologies involving angiotensin II. For example, it attenuates the hyperplastic effect of angiotensin II in cultured vascular smooth muscle cells [22], and reduces the age-related cardiac and vascular hypertrophy in the SHR [23]. In the renal and mesenteric vasculatures of hypertensive rats, DAA-I attenuates the vasoconstrictor effect of angiotensin II and III, an action mediated by AT_1R [24,25].

The present study was originally initiated to compare the effect of DAA-I on contractions to angiotensin II in WKY and SHR aortas. However, exploratory experiments revealed that the nonapeptide reduced angiotensin II-induced contractions only in SHR but not in WKY preparations (Fig. 1). The present experiments were designed to define the mechanism[s] underlying this difference.

2. Materials and methods

2.1. Animals

Male Wistar-Kyoto rats (WKYs) and spontaneously hypertensive rats (SHRs), 18 to 20 weeks old, were kept under controlled light (12 h:12 h light–dark cycle) and temperature (23 ± 1 °C) conditions. All rats used in the present study were born in the certified animal facility from BioLASCO (Yi-Lan Breeding Center, Taipei, Taiwan). The animals were fed with standard rat chow (Specialty Feeds Pty. Ltd., Glen Forrest, Australia) and had free access to tap water. All the experimental procedures were approved by the University of Malaya Animal Care and Ethics Committee.

2.2. Tissue preparation

The animals were sacrificed by cervical dislocation and the thoracic aorta was isolated and immediately placed in Krebs physiological salt solution (control solution) of the following composition (mM): NaCl, 118.9; $NaHCO_3$, 25.0; $MgSO_4$, 1.2; KCl, 4.7; KH_2PO_4, 1.0; glucose, 11.1 and $CaCl_2$, 2.4. The perivascular tissues were removed, and the aorta was cut into rings (3 to 4 mm long) that were suspended in jacketed organ chambers containing 5 ml of control solution, maintained at 37 °C and gassed with 95% O_2 and 5% CO_2. The rings were attached to isometric force transducers (Grass Instrument Co., Quincy, MA, USA). The transducer outputs were amplified and recorded continuously using a PowerLab recording system (AD Instrument, Sydney, NSW, Australia) connected to a portable computer display monitor. The rings were stretched to a baseline tension of 1 g and allowed to equilibrate for 45 min. In some experiments, the endothelium was removed by gently rubbing the lumen of the rings with a small forceps (Furchgott and Zawadzki, 1980). After stabilization, the tissues were primed three times with KCl (80 mM) to obtain a consistent reference contraction. The presence or absence of functional endothelium was verified prior to the actual experiment by determining whether or not relaxation occurred upon exposure to acetylcholine (10 μM), in phenylephrine (0.1 μM)-contracted preparations. Rings with more than 70% relaxation in response to acetylcholine were considered to have sufficient functional endothelium while those which relaxed less than 5% were accepted as rings without endothelium.

2.3. Experimental protocols

To examine the effect of DAA-I (0.001 pM–10 μM) on angiotensin II, concentration–contraction curves to angiotensin II were obtained in rings from WKY and SHR. The rings were incubated with captopril (30 μM) for 20 min to prevent the conversion of DAA-I to angiotensin III [26] prior to incubation with the nonapeptide for 30 min. Each concentration of DAA-I was studied in separate experiments.

To evaluate the role of the endothelium, the response to angiotensin II (0.1 nM–10 μM) was obtained in rings with or without endothelium from SHR aortas, in the absence or presence of DAA-I (0.1 μM, the most effective concentration). To determine the mechanism underlying the effect of DAA-I, the response to angiotensin II was compared in the presence of losartan (10 μM, AT_1R antagonist) [27], tempol (100 μM, a cell permeable superoxide dismutase (SOD) mimetic) [28], 1*H*-[1,2,4]-oxadiazolo [3,4-a]quinoxalin-1-one (ODQ, 3 μM, soluble guanylyl cyclase (sGC) inhibitor) [29] or apocynin (0.1 μM, an antioxidant and NAD(P)H oxidase inhibitor) [30]. Each inhibitor was added to the organ chamber at least 30 min prior to obtaining a concentration–response curve to angiotensin II.

2.4. Measurement of total nitrite/nitrate

Nitric oxide (NO) breaks down rapidly into nitrate (NO_3^-) and nitrite (NO_2^-) [31]. In order to measure NO products (total nitrate and nitrite, NO_x), isolated aortas were pre-incubated with captopril in the presence or absence of DAA-I (0.1 μM) for 30 min. The isolated aortic rings were also incubated with or without inhibitors (losartan, 10 μM and tempol, 100 μM). In addition, angiotensin II (0.1 μM) was added to all groups to mimic the experimental conditions of the organ chamber studies. Total NO_x were determined with a Nitrate/Nitrite Colorimetric Assay Kit from Cayman Chemical (Ann Arbor, MI, USA), following the manufacturer's instructions. Briefly, the tissues were homogenized in PBS (pH 7.4) using a glass tissue grinder (Wheaton™ Tenbroeck, New

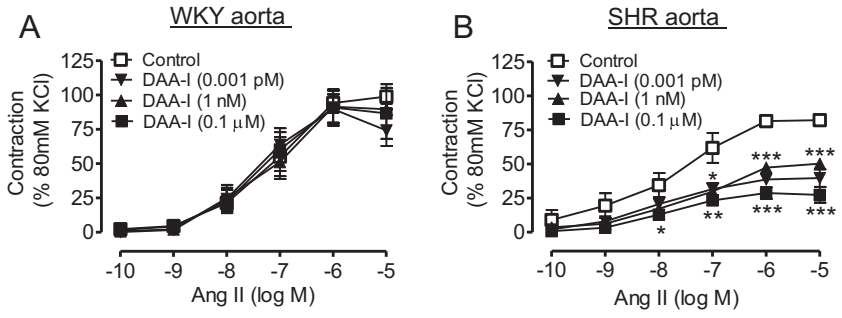

Fig. 1. Effect of DAA-I (0.001 pM–0.1 μM) on angiotensin II-induced contraction in isolated aortas of the WKY (A) and SHR (B). Results are shown as means ± S.E.M. (n = 5–7). $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with control.

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