Vascular Pharmacology 54 (2011) 29–35

Contents lists available at ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

Lack of heterologous receptor desensitization induced by angiotensin II type 1 receptor activation in isolated normal rat thoracic aorta

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ARTICLE INFO

Article history:
Received 24 September 2010
Received in revised form 8 November 2010
Accepted 23 November 2010

Keywords:
Angiotensin II
AT receptors
Desensitization
Rat aorta

ABSTRACT

We tested whether heterologous receptor desensitization induced by activation of AT_1 receptors may explain the purported relaxation produced by angiotensin II in normal rat aorta. Also, the role for AT_2 receptors in the promotion of vasodilation was studied. In endothelium-intact and endothelium-denuded aortic rings, angiotensin II elicited biphasic contractions, which were significantly depressed when repeated in each tissue. Angiotensin II produced biphasic responses on phenylephrine preconstricted endothelium-intact and endothelium-denuded tissues, without reducing precontractile tone. These responses were abolished in the presence of the AT_1 receptor antagonist losartan, but no relaxing responses to angiotensin II were uncovered. PD123319 did not influence angiotensin II responses in endothelium-intact tissues precontracted with phenylephrine; thus, under AT_2 receptors blockade the contractile effects of angiotensin II were not overexposed. In conclusion, angiotensin II-induced biphasic responses can be attributed to AT_1 receptors activation and rapid desensitization with time. Desensitization proved to be homologous in nature, since precontractile tone induced by phenylephrine was not depressed by angiotensin II (i.e., angiotensin II did not induce heterologous α_1-adrenergic receptors desensitization). We found no functional evidence of the participation of AT_2 receptors in angiotensin II elicited biphasic contractions. Angiotensin II does not exert relaxant effects in normal rat aorta.

1. Introduction

The renin–angiotensin system (RAS) plays a critical role in the control of cardiovascular and renal functions (Stroth and Unger, 1999). Angiotensin II (ANG II) as the main active peptide of the RAS acts at two G-protein coupled receptors (GPCR), ANG II type 1 (AT_1) and ANG II type 2 (AT_2) receptors; which have been defined on the basis of their differential pharmacological and biochemical properties (Inagami et al., 1994; Matsubara and Inada, 1998). At present time, it has been established beyond doubt that ANG II activation of AT_1 receptors induces vascular smooth muscle contraction, which leads to increased blood pressure. On the other hand, during the last years, evidence has accumulated that documents a physiological role for AT_2 receptors in the promotion of vasodilation in resistance (Baranov and Armstead, 2005; Batenburg et al., 2004; Hannan et al., 2003; Widdop et al., 2002) and capacitance vessels (Arun et al., 2004; Fukada et al., 2005; Tsutsumi et al., 1999). Since ANG II binds with similar affinity to AT_1 and AT_2 receptors (Carey and Siragy, 2003; Nouet and Nahmias, 2000) the corollary is that the vasoconstriction mediated by AT_1 receptors activation may be physiologically opposed by AT_2 receptors-induced vasodilation.

Considering in particular large-conductance vessels, current information suggests that AT_1 and AT_2 receptors may be functionally expressed in rat aorta. Lately, it has been shown that AT_2 receptors located in smooth muscle of normal rat aortic rings may mediate vasorelaxation via stimulation of the NO/cGMP pathway, vasodilator cyclooxygenase products, and voltage-dependent and Ca^{2+}-activated large-conductance K^+ channels (Fukada et al., 2005). Also, *another group* of researchers (Arun et al., 2004) reported that ANG II produced a concentration-dependent relaxation in endothelium-intact and endothelium-denuded rat thoracic aorta in the presence, but not in the absence, of AT_1 selective antagonists (losartan or valsartan). In addition, PD 123319 blocked the relaxation response to Ang II, suggesting that the response is mediated through AT_2 receptors (Arun et al., 2004). Importantly, there appears to be an up-regulation of AT_2 receptors in rat thoracic aorta under conditions associated with vascular tissue damage, such as diabetes and hypertension (Arun et al., 2004; Cosentino et al., 2005). On the other hand, it is well known that the contractile responses induced by ANG II in rat aorta manifest pronounced desensitization (Kuttan and Sim, 1993; Li et al., 1995; Sim and Kuttan, 1992). Several studies have shown that desensitization

Abbreviations: RAS, renin–angiotensin system; ANG II, angiotensin II; AT_1, angiotensin II type 1; AT_2, angiotensin II type 2; GPCR, G-protein coupled receptors; BK, bradykinin; NO, nitric oxide; cGMP, cyclic guanosin monophosphate; L-NAME, N^G-nitro-L-arginine methyl ester.

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doi:10.1016/j.vph.2010.11.002

(tachyphylaxis) to ANG II is associated with changes in both the affinity of the ligand for the AT_1 receptors and the coupling efficiency of the receptor system (Kuttan and Sim, 1993; Li et al., 1995; Sim and Kuttan, 1992). Desensitization to agonists, in particular to G-protein coupled receptor (GPCR)-agonists, is an extensively studied cellular process (Kelly et al., 2008; Kohout and Lefkowitz, 2003). Desensitization to GPCR-agonists can be homologous or heterologous in nature; homologous desensitization refers to the loss of response solely to agonists that act at a particular GPCR type, whereas heterologous desensitization refers to a broader effect involving the simultaneous loss of agonist responsiveness at multiple GPCR types (Kelly et al., 2008). Accordingly, vascular tissues precontracted with a GPCR-agonist may possibly show heterologous desensitization to another GPCR-agonist tested during the precontractile plateau.

To our knowledge, it has not been adequately studied if the relaxing response obtained with ANG II in precontracted normal rat aorta is the result of activating AT_2 receptors, exclusively (Arun et al., 2004; Fukada et al., 2005); or whether heterologous desensitization is an event responsible, at least in part, of the relaxant process (i.e., the vasorelaxation induced by ANG II might also reflect rapid heterologous desensitization of the GPCR that mediates the precontractile tone). Hence, the purpose of the present study was to investigate the relative participation of AT_2 receptors and heterologous receptor desensitization in the relaxation produced by ANG II in normal rat aorta.

2. Materials and methods

The experimental protocol was approved by the Animal Care and Use Committee of our Institution, and complied with the National Health and Medical Research Council of México guidelines. Male Wistar rats (250–300 g body weight; 10–12 weeks old) were housed under controlled conditions (22 ± 2 °C, 60% humidity and artificial light from 06:00 to 18:00 h). Normal chow and tap water were given ad libitum.

2.1. Tissue preparation and measurement of tension

Rats were anesthetized with pentobarbital sodium (50 mg/Kg, i.p.) and exsanguinated. The thoracic aortae were cleaned of fat and connective tissue, and cut into ring segments (4–5 mm in length). In some preparations, the endothelium was damaged by gently abrading the intimal surface of aortic rings with the tip of small forceps. Aortic rings were mounted in 10-ml tissue baths filled with physiological saline solution containing (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, EDTA 0.03, and dextrose 11.7. The medium was maintained at 37 °C, pH 7.4, and gassed continuously with 95% O_2 and 5% CO_2. Each tissue was placed under an initial resting tension of 2 g weight and equilibrated for 60 min prior to the execution of experimental protocols. Contractions were measured isometrically and recorded on a computer with the AcqKnowledge software (MP100WSW, Biopac Systems, Inc.; Santa Barbara, CA, USA). Tissues were primed by the addition of 1×10^{-6} M phenylephrine to the organ bath. After a steady-state contraction was achieved, bath contents were replaced with drug-free buffer several times. Tissues were then allowed to reach baseline tension and the priming procedure was repeated twice more before the execution of experimental protocols. Functional endothelium was checked by the presence of at least 80% relaxation in response to acetylcholine (1×10^{-6} M) after pre-constricting the tissues with phenylephrine (1×10^{-6} M). Otherwise, successful endothelial denudation was confirmed by the presence of small (lower than 10%) relaxations, or complete absence of relaxations, to acetylcholine.

2.2. Effects of ANG II on endothelium-intact and endothelium-denuded aortic rings

After equilibration, cumulative concentration–response curves for ANG II (1×10^{-10} to 1×10^{-5} M) were constructed in endothelium-intact and endothelium-denuded aortic ring preparations. Next, the rings were rinsed four times and re-equilibrated for 1 h. A second series of concentration–response curves for ANG II was then built. Cumulative concentration–response curves to ANG II were also constructed in endothelium-intact aortic rings after 30 min of incubation with the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM).

In separate experiments, the temporal course of a single low or high concentration of ANG II (either 1×10^{-8} or 1×10^{-5} M) was tested in endothelium-intact and endothelium-denuded tissues. Afterwards, the rings were rinsed four times and re-equilibrated for 1 h, and the same concentration of ANG II was administered again.

2.3. Cumulative concentration–response curves to ANG II under conditions of active tone induced by the α_1-adrenergic agonist phenylephrine

After the equilibration period, endothelium-intact and endothelium-denuded aortic rings were constricted with phenylephrine (1×10^{-7} M) and the contractions were allowed to stabilize. Subsequently, ANG II was applied cumulatively to obtained concentration–response curves. The effects of ANG II were tested in the presence or absence of selective AT_1 or AT_2 receptor blockers, losartan (100 nM) and PD123319 (1 μM), respectively. Each antagonist was applied 30 min before cumulative administration of ANG II. Only one cumulative concentration–response curve for ANG II was constructed in each tissue.

In other selective group of experiments, a sole concentration of ANG II (1×10^{-5} M) was tested—throughout 1 h—in endothelium-intact and endothelium-denuded aortic rings precontracted with phenylephrine (1×10^{-7} M).

In these sets of experiments with ANG II, vehicle (assay buffer)-treated rings served as temporal controls.

2.4. Effects of angiotensin receptors antagonists on the concentration–response curves to ANG II

Endothelium-intact and endothelium-denuded aortic rings were treated with different concentrations of losartan (10, 30, or 100 nM), PD123319 (0.1, 1, or 10 μM), or vehicle for 30 min. (Only one concentration of antagonist was tested in each tissue.) Then, concentration–response curves were established by exposing the aortic rings to increasing concentrations of ANG II until maximal contractile responses were observed (see data analysis). Only one cumulative concentration–response curve for ANG II was obtained in each aortic ring.

2.5. Drugs

The following drugs were used: L-phenylephrine hydrochloride, angiotensin II, acetylcholine chloride, L-NAME, losartan potassium, and PD123319 ditrifluoroacetate (Sigma Chemical Company; St. Louis, MO, USA). Drugs were dissolved in distilled water and subsequent dilutions made in assay buffer.

2.6. Data analysis

Results are expressed as the mean ± SEM for the number of aortic rings (n) obtained from four to six different animals. Contractions are expressed in grams (g) of developed force. Relaxations are expressed as the reduction of the maximum increment in tension (precontraction) obtained by phenylephrine addition. The negative logarithms of the molar concentrations of ANG II required to produce 50% of the maximal response ($-\log EC_{50}$) were calculated (by nonlinear regression analysis) for each individual concentration–response curve with a computer program (Graph Pad Software; San Diego, CA, USA). Agonist concentration ratios (CR) were determined from EC_{50} values and, where appropriate, estimates of pA_2 obtained from the equation: