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The effect of angiotensin 1–7 on tyrosine kinases activity in rat anterior pituitary

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Abstract

Angiotensin 1–7 (Ang 1–7) is a peptide originated from Ang II. It is known that in vessels Ang 1–7 shows opposite effects to Ang II. Ang 1–7 can modify processes of proliferation. However, Ang 1–7 action in pituitary gland cells was never studied. Moreover, the specific binding sites for Ang 1–7 are still unknown. The aim of this study was to examine the effects of Ang 1–7 on tyrosine kinases (PTKs) activity in the anterior pituitary. The reaction of phosphorylation was carrying out in presence of different concentration of Ang 1–7 and losartan (antagonist of AT1 receptor) and PD123319 (antagonist of AT2). Our results show that Ang 1–7 inhibited activity of PTK to 60% of basic activity. Losartan did not change the Ang 1–7-induced changes in PTKs activity. The presence of PD123319 together with Ang 1–7 caused stronger inhibition PTKs activity than Ang 1–7 alone. These observations suggest that Ang 1–7 binds to the novel, unknown, specific for this peptide receptor.

Keywords: Angiotensin 1–7; Tyrosine kinases; Losartan; PD123319; Angiotensin receptors

Angiotensin 1–7 (Ang-(1–7)) belongs to the family of angiotensin peptides comprising several biologically active compounds [1]. Ang-(1–7) is a seven amino acids peptide in most amounts originated from Ang II by action of carboxypeptidase, but also endopeptidase and locally by metalloendopeptidase [2–4]. This biologically active heptapeptide is particularly interesting because it can be formed by an ACE-independent pathway [2,3] and possesses selective angiotensinergic actions. Ang-(1–7) produces important central and peripheral effects similar to Ang II, including release of prostaglandins, NO, and vasopressin [5–7]. But some studies have indicated that this peptide counterregulates the cardiovascular actions of Ang II, acting as vasodilatory agent in many vascular beds [8–10]. Also unlike Ang II, Ang-(1–7) inhibits vascular smooth muscle growth [11]. It is possible that Ang-(1–7) may act through classical angiotensin receptors, AT1 and AT2. However, these opposing effects of Ang II and Ang-(1–7) occur at similar concentrations of them [12], suggesting the existence of specific receptor for Ang-(1–7), distinct from classical angiotensin II receptors, AT1 and AT2. The presence of a unique receptor, preferentially binding Ang-(1–7) was postulated by group of Ferrario from binding experiments on bovine aortic endothelial cells [1]. In anterior pituitary Ang II, acting via classical AT1 receptors, may modulate tyrosine kinases, and thus control proliferation of the cells [13]. Tyrosine kinases are coupled to the receptors of several growth factors and are involved in transduction of growth inducing signals [14]. It is known that Ang II, acting via classical AT1 receptors, may modulate tyrosine kinases in normal and tumoral pituitary gland [15,16]. However, so far there are no data about effects of Ang-(1–7) in anterior pituitary, as well as information about role of this peptide in control of cell

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proliferation. Therefore, we directly investigated the effect of Ang 1–7 on tyrosine kinase activity in anterior pituitary. Second, we examined the possibility of Ang-(1–7) action through classical AT1 and AT2 receptors, using their specific inhibitors, losartan and PD123, respectively.

Materials and methods

All used chemicals were of analytical grade. Most of them were received from Sigma, TCA was from Fluka. The radioactive γ-^{32}P-ATP was purchased from NEN (DuPont).

We used anterior lobe of pituitaries taken from intact 3-month-old Wistar male rats. The experimental procedure was approved by a Local Ethical Committee for Animal Experimentation. Tissues were homogenized in medium containing 0.32 M saecharose, 0.05 mM EDTA, 10 mM Tris/HCl, pH 7.4, 0.05 mM PMSF, and aprotinin 25 KIU/ml in 4 °C.

Samples were preincubated for 15 min in 37 °C with angiotensin 1–7 alone in several concentrations (10^{-6}–10^{-11} M) or with angiotensin 1–7 at the same concentrations in presence of losartan (10^{-8} M). In second study samples were preincubated with angiotensin 1–7 alone in concentrations 10^{-6}–10^{-11} M or with angiotensin 1–7 in presence of PD 123319 (10^{-8} M) at the same conditions. After some time the 0.1% Triton X-00 was added to all probes for 15 min (0 °C).

The reaction medium contained: 20 mM Tris/HCl, pH 7.4, 20 mM $MgCl_2$,1 mM $MnCl_2$, 1 mM EGTA, 0.5 mM EDTA, 0.1 M DDT, 1 mM ouabain, and 1 mM sodium orthovanadate [17].

The control group did not contain any tested compounds (basal activity). The reaction of phosphorylation was started after adding γ-[^{32}P]-ATP (200 μM) to mixture containing homogenate and artificial specific substrate for tyrosine kinase—poly GluTyr 4:1. Incubation was carried

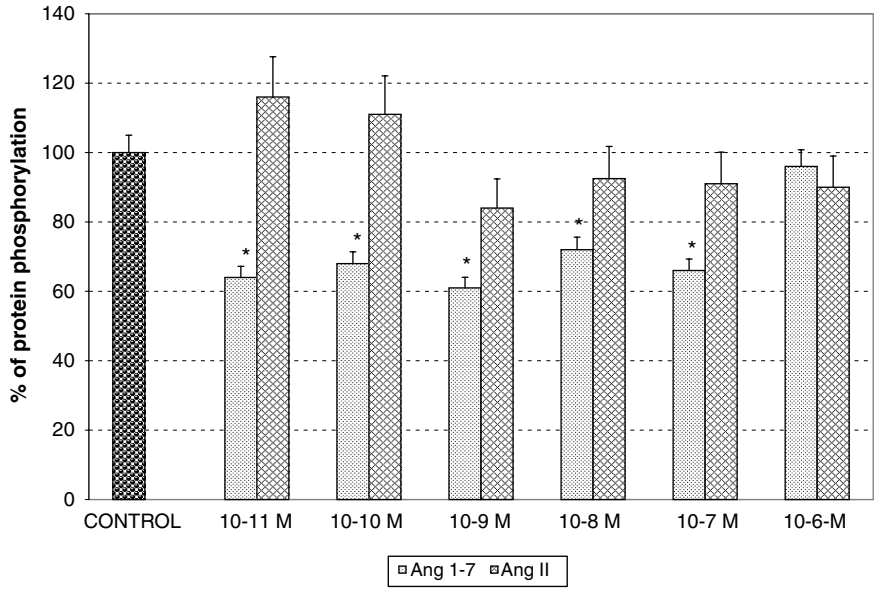

Fig. 1. The effect of angiotensin 1–7 in comparison to angiotensin II action on tyrosine kinases activity.

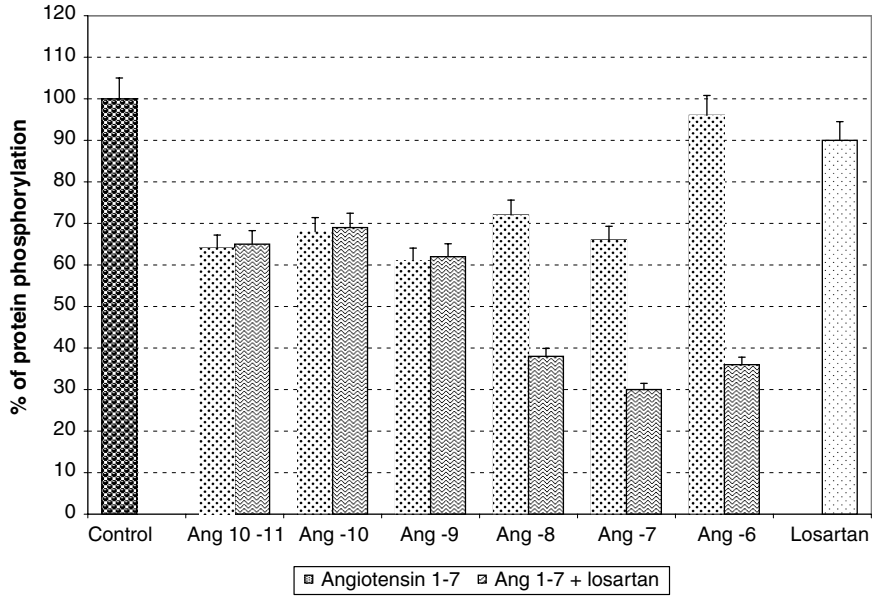

Fig. 2. The influence of specific inhibitor of AT1 receptor—losartan on angiotensin 1–7-induced changes in tyrosine kinases activity.