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Modulation of the $(Na^+ + K^+)$ATPase activity by Angiotensin-(1–7) in MDCK cells

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Abstract

In the present paper the effect of Ang-(1–7) on the distal tubule $(Na^+ + K^+)$ATPase activity was evaluated by using MDCK cells as a model. Confluent cell monolayers were incubated with increasing concentrations of Ang-(1–7) for 30 min. Thereafter, the $(Na^+ + K^+)$ATPase activity was evaluated and a dose–dependent (from 10^{-12} to 10^{-7} M) inhibition was observed. The maximal inhibitory effect (54%) was reached at the concentration of 10^{-8} M. The inhibitory effect of Ang-(1–7) was not affected by the AT_2 receptor selective antagonist PD123319 (from 10^{-10} to 10^{-7} M) but was blocked in a dose–dependent manner by the AT_1 receptor selective antagonists losartan (10^{-10} M), candesartan (10^{-17} M), irbesartan (2×10^{-12} M) and telmisartan (2×10^{-16} M). The signaling pathway triggered by stimulation of the AT_1 receptor was also investigated. The PI-phospholipase C (PI-PLC) inhibitor U73122 (5×10^{-8} M) blocked the inhibitory effect elicited by Ang-(1–7). Involvement of the protein kinase C (PKC) was evidenced by the sensitivity of the inhibitory effect of Ang-(1–7) to calphostin C (6.32×10^{-7} M) and the lack of additive effects when the cells were co-incubated with Ang-(1–7) and 3.2×10^{-8} M PMA. Altogether, these results demonstrate that Ang-(1–7) inhibits the $(Na^+ + K^+)$ATPase activity of the prototypic distal tubule cell MDCK through the AT_1 receptor-mediated stimulation of PI-PLC/PKC signaling pathway.

Keywords: $(Na^+ + K^+)$-ATPase; Angiotensin-(1–7); AT_1 receptor; MDCK cells

1. Introduction

It is well established that Ang-(1–7) plays an important role in maintaining body fluid and electrolyte balance as well as long-term blood pressure homeostasis [1]. These effects are, at least in part, mediated by its action on the kidney [2,3]. Furthermore, it has been demonstrated that kidney may be a primary site for Ang-(1–7) synthesis [4]. While natriuretic and diuretic effects have been attributed to Ang-(1–7), opposing effects have been related to Ang II [3,5]. Regulation of the renal sodium excretion by Ang-(1–7) has been associated to the modulation of sodium transporters in both proximal and distal segments of the nephron [2,6]. Nevertheless, neither the identities of these sodium transporters nor the molecular mechanisms involved in the modulation are well known.

The transcellular sodium reabsorption is largely driven by the primary active sodium transporter localized in the basolateral membrane of epithelial tubular cells, the ouabain-sensitive $(Na^+ + K^+)$ATPase [7]. This enzyme is an important target for several hormones and autacoids that modulate renal sodium excretion [7,8]. Although, a significant reduction induced by Ang-(1–7) in the transport-

Abbreviations: Angiotensin II, Ang II; Angiotensin-(1–7), Ang-(1–7); ATP, Adenosine triphosphate (sodium salt); Calphostin C, Cph; EDTA, Ethylenediaminetetraacetic acid; HEPES, (*N*-2-hydroxyethylpiperazine *N*'-2-ethanesulfonic acid); PMA, Phorbol 12-myristate 13-acetate; PMSF, Phenylmethylsulfonyl fluoride; Tris, Tris(trishydroxymethyl)–aminomethane.

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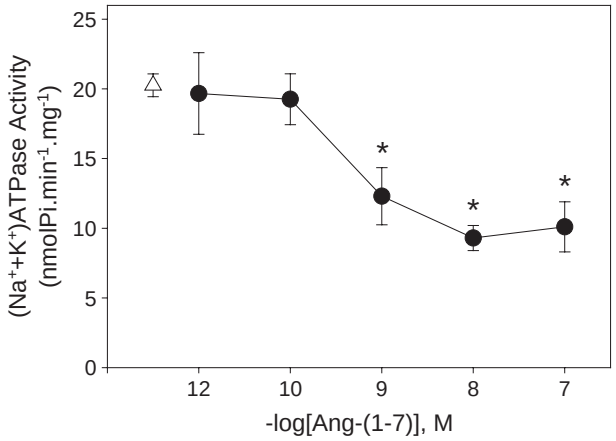

Fig. 1. Inhibition of the $(Na^+ + K^+)$ATPase activity of MDCK cells by Ang-(1–7). Confluent cultures of MDCK cells were incubated with different Ang-(1–7) concentrations (from 10^{-12} to 10^{-7} M) for 30 min at 37 °C. The $(Na^+ + K^+)$ATPase activity of the cell homogenates was measured as described in Materials and methods. Results are expressed as absolute values ± SE. *Statistically significant when compared to control ($p < 0.05$) ($n = 8$).

dependent O_2 consumption in the proximal tubule has been previously reported [2], suggesting a role for the $(Na^+ + K^+)$ATPase as the target, a straightforward investigation demonstrating the modulation of this sodium transporter by Ang-(1–7) is still lacking, particularly in the distal segment of the nephron.

It has been demonstrated that the effects of Ang-(1–7) are mediated by G-protein coupled receptors (GPCR), such as the AT_1 and AT_2 receptors [3]. The signaling pathway triggered by interaction of Ang-(1–7) with these receptors modulates different protein kinase activities [9–11]. Modulation of the $(Na^+ + K^+)$ATPase activity through phosphorylation of the α-subunit by PKC and PKA has been previously demonstrated in purified preparations as well as intact cells and tissue homogenates [12,13].

The aim of the present work is to study the modulation of the $(Na^+ + K^+)$ATPase by Ang-(1–7) in the distal tubule MDCK cells (an epithelial-derived cell line from canine kidney distal nephron). Our results demonstrate that the AT_1 receptor mediates the inhibitory effect of Ang-(1–7) on the $(Na^+ + K^+)$ATPase activity. Furthermore, the inhibitory response elicited by Ang-(1–7) involves the PI-PLCβ/PKC signaling pathway.

2. Materials and methods

2.1. Chemicals and reagents

ATP, ouabain, HEPES, Tris, Ang-(1–7) (Asp-Arg-Val-Tyr-Ile-His-Pro), PD123319, calphostin C, were purchased from Sigma Chemical, St. Louis, MO, USA. U73122 (1-[6((17b-3-methoxyestra-1,3,5(10)-trien-17-yl) amino hexyl]-1*H*-pyrrole-2,5-dione) was purchased from Calbiochem, USA. The AT_1 selective-antagonists used were: Losartan (Medley S.A., Brazil), Candesartan (AstraZeneca AB Sodertalje, Sweden), Irbesartan (Bristol-Myers Squibb Pharmaceutical Group, EUA).

All other chemical reagents used in this work were of the highest purity available. All solutions were prepared with deionized glass-distilled water. [^{32}P]Pi was obtained from the Institute of Energetic and Nuclear Research, Brazil. [γ-^{32}P]ATP was prepared as described by Maia et al. [14].

2.2. Cell culture

The epithelial-derived cell line from canine kidney distal tubule, MDCK, purchased from Rio de Janeiro Cell Bank/UFRJ, was grown in monolayer cultures in Dulbecco's modified Eagle's medium (Gibco BRL) containing 10% fetal bovine serum (purchased from Gibco-BRL), 0.15% sodium bicarbonate, 1% Antibiotic–Antimycotic (prepared with 10,000 U/mL penicillin, 10,000 μg/mL streptomycin, 25 μg/mL fungizone, purchased from Gibco-BRL). Cell cultures were incubated at 37 °C in humidified-air containing 5% CO_2.

2.3. Cell treatments

Confluent cultures of MDCK cells grown in 6 well-plates were washed with PBS and incubated with 2 mL of culture medium for 6 h at 37 °C. After this period, Ang-(1–7) and other testing drugs were added to the culture medium and the cells were further incubated for 30 min. Thereafter, the cell monolayers were washed with PBS and harvested from the

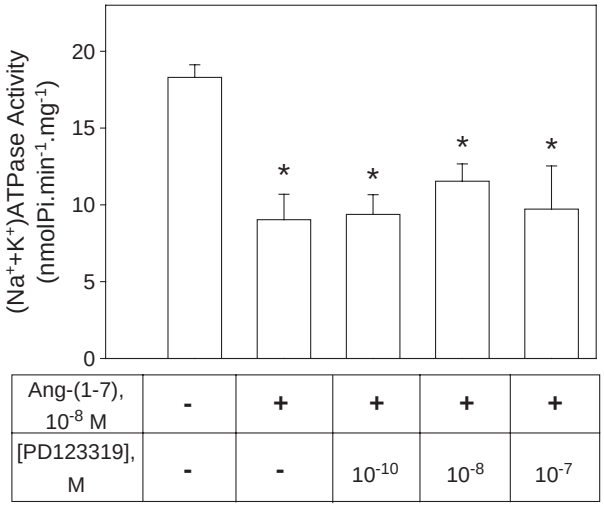

Fig. 2. Inhibition of the $(Na^+ + K^+)$ATPase activity by Ang-(1–7) is not affected by the AT_2 receptor selective antagonist PD123319. Confluent cultures of MDCK cells were incubated with Ang-(1–7) for 30 min at 37 °C in the absence and in the presence of PD123319 (from 10^{-10} to 10^{-7} M). The $(Na^+ + K^+)$ATPase activity of the cell homogenates was measured as described in Materials and methods. PD123319 (10^{-8} M) alone did not modify the $(Na^+ + K^+)$-ATPase activity. Results are expressed as absolute values ± SE. *Statistically significant when compared to control ($p < 0.05$) ($n = 13$).