Short Communication

Angiotensin II Induces Modulation of Calcium Channel Currents in Osteoblasts

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Abstract

Angiotensin II (Ang II) plays a major role in the maintenance of extracellular fluid volume and blood pressure. In addition to its well-established role in circulatory homeostasis, it has been implicated in the process of bone formation. Osteoblasts play a major role in bone formation, employing intracellular Ca^{2+} as a second messenger to modulate hormonal responses and as a cofactor for mineralization. Voltage-dependent Ca^{2+} channels (VDCCs) mediate the influx of Ca^{2+} in response to membrane depolarization. The purpose of this study was to investigate the effects of Ang II on VDCC currents in osteoblasts using a patch-clamp recording method. To our knowledge, the data presented here demonstrate for the first time that Ang II facilitates VDCCs in osteoblasts.

Key words: Angiotensin II—Osteoblast—Voltage-dependent Ca^{2+} channels—Patch clamp experiment

Introduction

In cells of osteoblastic lineage, intracellular Ca^{2+} signals coordinate cell behavior, which translates into systemic control of Ca^{2+} homeostasis and maintenance of normal bone mineral density. The action of the renin-angiotensin system (RAS) differs depending on the biological processes involved, ranging from development and reproduction to car-

diovascular and renal functions. These actions are mainly mediated by the octapeptide hormone Angiotensin II (Ang II), which interacts with seven-transmembrane G-protein coupled receptors. Recently, it has been suggested that Ang II affects bone cells. For example, Hiruma *et al.* reported that Ang II stimulated proliferation of osteoblast-rich populations from rat calvariae⁹⁾. Voltage-dependent Ca^{2+} channels (VDCCs) serve as crucial mediators

of membrane excitability¹²⁾ and many Ca^{2+} -dependent functions such as growth of bone⁴⁾, regulation of proliferation¹¹⁾, enzyme activity¹⁵⁾ and gene expression¹³⁾. Modulation of VDCCs by AngII in various types of cell has been described previously^{5,10)}. However, the effect of AngII in osteoblasts remains to be clarified. Therefore, the purpose of this study was to investigate the effects of AngII on VDCC currents (I_{Ca}) in osteoblasts.

Materials and Methods

Murine osteoblastic MC3T3-E1 cells were purchased from Summit Pharmaceuticals International Corporation (Tokyo, Japan). Cells were cultured at 37°C in a 5% (v/v) CO_2 atmosphere with α -modified minimal essential medium (α -MEM; Gibco BRL, Grand Island, NY, U.S.A.). Unless otherwise specified, the medium contained 10% (v/v) heat-inactivated fetal bovine serum, 100 U/ml penicillin and 100 mg/ml streptomycin. Cell culture medium was changed every 2–3 days. For patch-clamp experiments, cells were harvested using a 0.05% trypsin/0.02% EDTA solution after reaching confluence. Cells were plated at very low density in 35-mm tissue culture dishes. Prior to recordings, the cells were washed at least three times with Krebs solution of the following composition (in mM): 136 NaCl; 5 KCl; 2.5 CaCl_2 ; 0.5 MgCl_2 ; 10.9 glucose; 11.9 NaHCO_3 and 1.1 NaH_2PO_4 . The pH was 7.3–7.4. Cell culture reagents were purchased from Sigma (Tokyo, Japan).

Voltage-clamp recordings were obtained using whole-cell configuration patch-clamp technique⁸⁾. Fabricated recording pipettes (2–3 M Ω) were filled with internal solution of the following composition (in mM): 150 CsCl, 5 EGTA, 10 D-glucose, and 10 HEPES. The pH was adjusted to 7.3 with CsOH. After the formation of a giga seal, in order to record I_{Ca} carried by Ba^{2+} (I_{Ba}), the extracellular solution was replaced replacing Krebs solution with a solution containing the following (in mM): 115 BaCl_2 and 20 HEPES. The pH was adjusted to 7.4 with tetraethylammo-

nium hydroxide. Command voltage protocols were generated with the computer software pCLAMP version 10 (Axon Instruments, Union City, CA, U.S.A.) and transformed to an analogue signal using the DigiData1440A interface (Axon Instruments). The command pulses were applied to the cells through an L/M-EPC7 amplifier (HEKA Elektronik, Lambrecht, Germany). The currents were recorded with an amplifier and the computer software pCLAMP10 acquisition system. Access resistance (<15 M Ω) was determined by transient responses to voltage commands. AngII was purchased from Sigma. AngII was dissolved in distilled water, and was diluted to the desired final concentration in the external solution just before use.

Results

The properties of VDCCs in osteoblasts have been demonstrated previously using current-voltage relationships²⁰⁾. Osteoblasts have L-type VDCCs, which are fully activated at a test potential ($= -0$ mV) from a holding potential ($= -80$ mV). Therefore, in this study, full activation of I_{Ba} was obtained by applying a test pulse from a holding potential of -80 mV in depolarizing voltage steps of -0 mV evoked every 20 sec.

Representative examples of superimposed I_{Ba} traces in the absence or presence of $1 \mu\text{M}$ AngII are shown in Fig. 1A. As shown in Fig. 1, application of $1 \mu\text{M}$ AngII rapidly and reversibly facilitated I_{Ba} from -64 to -84 pA (31% facilitation, $n = 1$) in this cell.

To investigate the voltage dependency of facilitation of I_{Ba} by AngII, we analyzed the current-voltage relations in the absence or presence of AngII. The current-voltage relations measured before and during application of AngII are shown in Fig. 1B. From a holding potential of -80 mV, I_{Ba} was activated with a peak current amplitude at 0 mV. AngII did not alter the current-voltage relationship.

Fig. 1

- A: Typical superimposed I_{Ba} traces in absence and presence of $1\mu\text{M}$ AngII. I_{Ba} was evoked from holding potential of -80 mV in 100-msec voltage steps to 0 mV at 20-sec intervals.
- B: Current-voltage relationship of I_{Ba} evoked by a series of voltage steps from holding potential of -80 mV to test potentials between -80 and $+50\text{ mV}$ in $+10\text{ mV}$ increments in absence (opened circles) and presence (filled circles) of $1\mu\text{M}$ AngII.

Discussion

The results of this study showed that AngII facilitated VDCCs in osteoblasts. However, only the effect of $1\mu\text{M}$ AngII on I_{Ba} was investigated here, and further study is needed to elucidate the effect at various concentrations.

Osteoblasts express AngII receptors¹⁴. It has also been reported that AngII induces bone resorption, suppresses alkaline phosphatase activity *in vitro*, and stimulates the proliferation of osteoblasts^{1,6}.

In addition, AngII has been shown to act on osteoblasts and stimulate the production of the osteoclastogenic cytokine receptor activator of NF- κ B ligand (RANKL),

thereby indirectly stimulating differentiation of osteoclasts^{2,18}.

It has been also reported that AngII decreases the level of osteocalcin mRNA, which is a well-known marker of osteoblastic differentiation⁷.

Pharmacological evidences has defined two types of AngII receptor, designated AT₁ and AT₂^{3,19}. Hagiwara *et al.* demonstrated that AngII influenced differentiation of osteoblastic cells *via* AT₁ receptors⁷.

In general, it is believed that AngII elicits increases in sympathetic outflow and blood pressure *via* AT receptors in the central nervous system (CNS). Recent studies, however, have demonstrated that the actions of AngII also another facet. For example, several studies have demonstrated that AngII-induced activation of microglia and production of inflammatory cytokines in the CNS, which contributed to an increase in blood pressure, possibly *via* a paracrine stimulatory action on nearby sympathetic efferent neurons^{16,17}. Therefore, further study is needed to elucidate the precise mechanism underlying the facilitation of I_{Ba} by AngII in osteoblasts.

Bone is rich in blood vessels and bone formation requires vascularization, suggesting interactions between bone cells and vasoactive peptides in the local environment. The present results suggest that AngII is a novel local regulator of bone remodeling.

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