Short Communication

Adrenomedullin Facilitates Calcium Channel Currents in Osteoblasts

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Abstract

Osteoblasts play a major role in bone formation. Osteoblasts employ intracellular Ca^{2+} as a second messenger to modulate hormonal responses and a cofactor for bone mineralization. Adrenomedullin (ADM) promotes osteoblast growth and proliferation, inducing an increase in bone mass. Voltage-dependent Ca^{2+} channels (VDCCs) mediate the influx of Ca^{2+} in response to membrane depolarization. Voltage-dependent Ca^{2+} channels serve as crucial mediators of many Ca^{2+} -dependent functions, including growth of bone and regulation of proliferation. The purpose of this study was to investigate the effects of ADM on VDCC currents in osteoblasts using a patch-clamp recording method. To our knowledge, the data presented here demonstrate for the first time that ADM facilitates VDCCs in osteoblasts.

Key words: Adrenomedullin — 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. Adrenomedullin (ADM) is a 52-amino-acid peptide originally isolated from a human pheochromocytoma¹²⁾. It is structurally and functionally related to calcitonin gene-related peptide (CGRP) and belongs to the amylin peptide family¹⁴⁾. Dorit

et al. demonstrated that ADM receptors are expressed in osteoblasts⁶⁾. Several studies have shown that ADM stimulate osteoblast proliferation^{2,3,9)}. Voltage-dependent Ca^{2+} channels (VDCCs) serve as crucial mediators of membrane excitability¹⁵⁾ and many Ca^{2+} -dependent functions such as growth of bone⁷⁾, regulation of proliferation¹³⁾, enzyme activity¹⁷⁾ and gene expression¹⁶⁾. Modulation of VDCCs by CGRP and ADM in various types of cells has been described previously^{8,11,19)}. However, the effect of ADM on VDCCs in osteoblasts remains to

be clarified. Therefore, the purpose of this study was to investigate the effects of ADM 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) CO₂ 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, when cells reached 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 CaCl₂; 0.5 MgCl₂; 10.9 glucose; 11.9 NaHCO₃ and 1.1 NaH₂PO₄. 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 technique¹⁰. 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 Ba²⁺ (I_{Ba}), the extracellular solution was replaced changing Krebs solution for a solution containing the following (in mM): 115 BaCl₂ and 20 HEPES. The pH was adjusted to 7.4 with tetraethylammonium hydroxide (TEA-OH). 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. Adrenomedullin was purchased from Sigma.

Results

The properties of VDCCs in osteoblasts using current-voltage relationships have been demonstrated previously¹⁸. Osteoblasts have L-type VDCCs, which are fully activated at a test potential (= -10 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 -10 mV evoked every 20 sec.

Representative examples of superimposed I_{Ba} traces in the absence or presence of 1 μ M ADM are shown in Figs. 1A and B. As shown in Figs. 1A and B, application of 1 μ M ADM rapidly and reversibly facilitated I_{Ba} from -49 pA to -127 pA (159% facilitation) in this cell. Representative examples of superimposed I_{Ba} traces in the absence or presence of 0.1 μ M ADM are shown in Figs. 1C and D. As shown in Figs. 1C and D, application of 0.1 μ M ADM rapidly and reversibly facilitated I_{Ba} from -78 pA to -93 pA (19% facilitation) in this cell.

Discussion

The results of this study showed that ADM facilitates VDCCs in osteoblasts.

Cornish *et al.* has suggested that ADM stimulate osteoblast proliferation and increases intracellular Ca²⁺ levels ([Ca²⁺]_i)⁴. It is possible that ADM-induced proliferation is due to an increase in [Ca²⁺]_i by facilitation of VDCCs.

There are several mechanisms of VDCCs facilitation⁵. L-type VDCCs facilitation can result from a strong conditioning depolari-

Fig. 1 A: Typical superimposed I_{Ba} traces according to time course graph B. Panel A shows corresponding current traces indicated by numbers (1 and 2) in B. I_{Ba} was evoked from holding potential of -80 mV in 100-msec voltage steps to -10 mV at 20-sec intervals. B: Typical time course of ADM-induced modulation of I_{Ba} . ADM ($1 \mu\text{M}$) was bath-applied during times indicated by black bar. C: Typical superimposed I_{Ba} traces according to time course graph D. Panel C shows corresponding current traces indicated by numbers (3 and 4) in D. D: Typical time course of ADM-induced modulation of I_{Ba} . ADM ($0.1 \mu\text{M}$) was bath-applied during times indicated by black bar.

zation that recruits silent channels¹⁾. Alternatively, L-type VDCCs can be facilitated by protein kinases¹⁴⁾. L-type VDCCs possess several consensus protein kinase A (PKA) and protein kinase C (PKC) phosphorylation sites and physiological studies have demonstrated channel facilitation by these enzymes¹⁴⁾. We previously demonstrated that ADM facilitates VDCCs mediated by PKA in submandibular ganglion⁸⁾. We have also demonstrated that ADM facilitates VDCCs involving mitogen-activated protein kinase in nucleus tractus solitarius¹¹⁾. It is possible that ADM could play a role in therapy for osteoporosis. Therefore, ADM receptor's intracellular pathways in

osteoblasts should be investigated in further study.

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