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Short Communication

Prepulse Facilitation of Calcium Channel Current in Osteoblasts

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Abstract

Osteoblasts play a major role in bone formation. Osteoblasts employ intracellular Ca$^{2+}$ as a second messenger modulating hormonal responses and a cofactor for bone mineralization. Voltage-dependent Ca$^{2+}$ channels (VDCCs) are most commonly present in excitable cell membranes. They are also present at lower levels even in most non-excitable cells too. In both types of cell, they mediate the influx of Ca$^{2+}$ in response to membrane depolarization. Prepulse facilitation is a phenomenon in which a train of depolarization, or a long and strong depolarizing pulse, induces a form of VDCC that exhibits an increased opening probability in response to a given test potential; and this persists for several seconds after repolarization. We believe this to be the first study to demonstrate that strong depolarization prepulses both increase and decrease VDCCs in osteoblasts.

Key words: Osteoblast — Voltage-dependent Ca$^{2+}$ channels — Prepulse facilitation — 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 maintains normal bone mineral density.

Voltage-dependent Ca$^{2+}$ channels (VDCCs) serve as crucial mediators of membrane excitability$^{[14]}$ and many Ca$^{2+}$-dependent functions such as growth of bone$^{[9]}$, regulation of proliferation$^{[15]}$, enzyme activity$^{[16]}$ and gene expression$^{[17]}$. The key cell surface sensor of mechanical transduction and control of intracellular Ca$^{2+}$ concentration in osteoblasts is the L-type VDCC.

Prepulse facilitation is a phenomenon in which a train of depolarization, or a long and strong depolarizing pulse, induces a form of VDCC that exhibits an increased opening probability in response to a given test potential; and this persists for several seconds after repolarization$^{[19]}$. However, prepulse facilitation of VDCCs in osteoblasts has not been clarified. Consequently, the purpose of this study was to investigate prepulse facilitation...
of VDCCs in osteoblasts.

Materials and Methods

Murine osteoblastic MC3T3-E1 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 (FBS), 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 technique²⁵. Fabricated recording pipettes (2–3 MΩ) 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 VDCC current (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 TEA-OH. Command voltage protocols were generated with a 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 MΩ) was determined by transient responses to voltage commands.

Results

Representative examples of superimposed I_Ba traces are shown in Fig. 1A and B. To investigate the prepulse facilitation of I_Ba, we used a double-pulse voltage protocol as shown in Fig. 1. Paired I_Ba were evoked from holding potential of −80 mV by 100 ms voltage step to 0 mV. Intervening strong depolarizing prepulse (100 mV, 30 ms) ended 5 ms prior to second I_Ba.
increased the \( I_{\text{Ba}} \), the amplitude of \( I_{\text{Ba}} \) was 70.3 ± 67.8 pA (first \( I_{\text{Ba}} \)) to 127.0 ± 61.6 pA (second \( I_{\text{Ba}} \)), \( n = 6 \).

In contrast, as shown in Fig. 1B, a strong depolarization prepulse decreased the amplitude of \( I_{\text{Ba}} \) from −170 pA (first \( I_{\text{Ba}} \)) to −70 pA (second \( I_{\text{Ba}} \)) in other cells. When the prepulse decreased the \( I_{\text{Ba}} \), the amplitude of \( I_{\text{Ba}} \) was 159.2 ± 27.1 pA (first \( I_{\text{Ba}} \)) to 99.7 ± 28.9 pA (second \( I_{\text{Ba}} \)), \( n = 4 \).

**Discussion**

In this study, it was demonstrated that a strong depolarization prepulse both increased and decreased \( I_{\text{Ba}} \) in osteoblasts. We believe this to be the first study to report that depolarization of prepulses decrease \( I_{\text{Ba}} \) in osteoblasts.

There are at least two distinct forms of prepulse facilitation of VDCCs. One type, prepulse facilitation of N- and P/Q-type VDCCs, is mediated by direct interactions between GTP-binding protein (G-protein) \( \beta \gamma \) subunits and VDCCs \(^7,^{18}\). In this model, VDCCs are tonically inhibited by G-proteins \( \beta \gamma \) subunits \(^6,^{10}\). Application of a prepulse may release G-proteins \( \beta \gamma \) subunits from VDCCs.

Another type, prepulse facilitation of L-type VDCCs of thalamic neurons, is independent of G-proteins and phosphorylation \(^11\). Similar to previous suggestions that direct conformational changes in L-type VDCCs underlies the mechanism for voltage-dependent facilitation in smooth muscle L-type VDCCs \(^3\), these authors concluded that no chemical modification is necessary to produce the facilitated state of VDCCs.

A possible explanation for the discrepancy in these results is that the contribution of different splice variants of VDCCs \( \alpha \) and \( \beta \) subunits may affect the kinetic properties of \( I_{\text{Ba}} \). VDCCs are hetero-oligomers consisting of a number of subunits: \( \alpha \) subunits, the pore-forming subunit; and several accessory subunits, including \( \alpha \delta \) and \( \beta \) subunits. It has been demonstrated that prepulse facilitation of \( I_{\text{Ba}} \) was observed in HEK293 cells expressing the \( \alpha \) subunit, the \( \alpha \beta \) complex, but not in cells expressing the \( \alpha \beta \alpha \delta \) complex \(^12\). In osteoblasts, L-type VDCCs are composed of the \( \alpha \), \( \alpha \delta \) and \( \beta \) subunits but are devoid of a \( \gamma \)-subunit \(^9\).

It is assumed that VDCCs exist in two modes (i.e., different gating patterns): the “willing” mode and the “reluctant” mode. In the willing mode, VDCCs are opened readily by small depolarizations (first \( I_{\text{Ba}} \)), whereas in the reluctant mode, VDCCs require much larger depolarizations to open (second \( I_{\text{Ba}} \)) \(^1\). In addition, it has been suggested that G-protein can inhibit VDCCs (tonic inhibition) \(^9\). It has been reported that a strong depolarization prepulse relieves the tonic inhibition of VDCCs produced by G-protein activation \(^7\). We believe that the presence of the increasing versus decreasing response on \( I_{\text{Ba}} \) is more likely due to expression of different G-proteins and/or channel modes. The kinetic mechanisms of VDCCs must be investigated in more detail in further study.

**References**

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