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Interleukin-1β and 6-induced calcium channel current modulation in MC3T3-E1 cells

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Keywords: Osteoblasts; Interleukin-1β; Interleukin-6; calcium channels
Abstract

Interleukin-1β (IL-1β) and -6 (IL-6) are inflammatory cytokines that are involved in bone resorption under pathological conditions. Recently, the cytokines are also demonstrated that they involved in bone formation under physiological conditions. Osteoblasts are known to play a major role in the bone formation. Voltage sensitive Ca\(^{2+}\) channels (VSCCs) serve as crucial mediators of membrane excitability and many Ca\(^{2+}\)-dependent functions such as growth of bone, regulate proliferation, differentiation, enzyme activity and gene expression. Several studies demonstrated that IL-1β and IL-6 modulated VSCCs in neurons. Previously, we have been reported that hormones and peptides modulated VSCCs in pre-osteoblast cell line MC3T3-E1. In this study, the effects of IL-1β and -6 on VSCCs current (I_{Ca}) carried by Ba\(^{2+}\) (I_{Ba}) in MC3T3-E1 were investigated using patch-clamp recording. Application of 50 pM, 500 pM 5 nM and 50 nM IL-1β facilitated I_{Ba} 22.8 ± 4.0%, 21.7 ± 2.0%, 34.1 ± 3.9% and 56.7 ± 5.1%, respectively. Application of 50 pM, 500 pM and 5 nM IL-6 facilitated I_{Ba} 15.8±2.7%, 22.9±4.5% and 35.6±4.0%, respectively. In contrast, 50 nM IL-6 inhibited I_{Ba} 21.9±3.4%. In conclusion, IL-1β facilitated VSCCs in MC3T3-E1 cells. In addition, we acquired two different groups of results in IL-6: IL-6-induced facilitation and inhibition of VSCCs in MC3T3-E1 cells. We considered that such biphasic effects are depends on IL-6 receptor's intracellular signals transduction mechanisms.
1. Introduction

Interleukin-1β (IL-1β), a potent inflammatory cytokine, is upregulated during inflammation mainly produced by activated monocytes and macrophages [1,2]. It stimulates bone resorption in several cell types, including osteoblasts [3]. IL-6 is a multifunctional cytokine which has diverse effects on bone metabolism. IL-6 has been shown to affect growth and differentiation in osteoblasts [4-6].

Osteoblasts are known to play a major role in the bone formation. It is well accepted that osteoblasts express IL-1β and -6 receptors and therefore it plays an important role in bone remodeling [7,8].

Voltage sensitive Ca²⁺ channels (VSCCs) serve as crucial mediators of membrane excitability [9] and many Ca²⁺-dependent functions such as growth of bone [10], regulate proliferation [11], differentiation [12], enzyme activity [13] and gene expression [14]. It also has been demonstrated that osteoblasts express VSCCs [15,16]. Several reports demonstrated that IL-1β and -6 modulated VSCCs in neuronal cells [17,18]. However, the mechanism of IL-1β and -6 effects on VSCCs in osteoblasts remains unclear and even controversial.

Consequently, in this study we investigated the effect of IL-1β and -6 on VSCCs in osteoblast cell line (MC3T3-E1).

2. Materials and Methods

2.1. Cell Culture
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 (Mo, U.S.A). Our recordings were performed on 10-30 μm in diameter, since they were well suited for patch clamp recordings.

2.2. Whole-cell patch-clamp recordings

Voltage-clamp recordings were conducted using the whole-cell configuration of the patch-clamp technique [19]. Fabricated recording pipettes (2-3 MΩ) were filled with the 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 VSCCs current (I_Ca) carried by Ba²⁺ (I_Ba), the extracellular solution was replaced from Krebs solution to a solution containing the following (in mM): 115 BaCl₂ and 20 HEPES. The pH was adjusted to 7.4 with tetraethylammonium hydroxide. 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 a DigiData1440A interface (Axon Instruments, Union City, CA, U.S.A.). The command pulses were applied to cells through an L/M-EPC7 amplifier (HEKA Elektronik, Lambrecht, Germany). The currents were recorded with the amplifier and a computer software pCLAMP10 acquisition system. Access resistance (<15 MΩ) was determined by transient responses to voltage commands.

2.3. Chemicals

IL-1β and 6 were purchased from Sigma (Saint Louis, MO, U.S.A.).

2.4. Analysis

All data analyses were performed using the pCLAMP10 acquisition system. Values in text and figures are expressed as mean ± S.E.M.

3. Results

3.1. IL-1β facilitated I_{Ba}

Fig. 1A and B shows that, in the presence of high external Ba^2+, 5 nM IL-1β facilitated I_{Ba} as shown with raw current traces obtained at +10 mV from −80 mV in the absence and presence of IL-1β. I_{Ba} was evoked every 5 s with a 100 ms depolarizing voltage step to +10 mV from a holding potential of −80 mV. As shown in Fig. 1A, application of 5
nM IL-1β facilitated $I_{Ba}$ from $-161$ pA to $-225$ pA (39.7% facilitation) in this cell. To investigate the voltage dependency of facilitation of $I_{Ba}$ by IL-1β, we analyzed the current-voltage relations in the absence and presence of IL-1β. The current-voltage relations measured before and during application of IL-1β are shown in Fig. 1C. From a holding potential of $-80$ mV, $I_{Ba}$ was activated with a peak current amplitude at $+20$ mV. IL-1β did not alter the current-voltage relationship. These results suggest that 5 nM IL-1β facilitated $I_{Ba}$ in MC3T3-E1 cells.

3.2. IL-6 facilitated $I_{Ba}$

Fig. 2A and B shows that 5 nM IL-6 facilitated $I_{Ba}$ as shown with raw current traces obtained at $+10$ mV from $-80$ mV in the absence and presence of IL-6. $I_{Ba}$ was evoked every 5 s with a 100 ms depolarizing voltage step to $+10$ mV from a holding potential of $-80$ mV. As shown in Fig. 2A, application of 5 nM IL-6 facilitated $I_{Ba}$ from $-75$ pA to $-103$ pA (37.3% facilitation) in this cell.

To investigate the voltage dependency of facilitation of $I_{Ba}$ by 5 nM IL-6, we analyzed the current-voltage relations in the absence and presence of 5 nM IL-6. The current-voltage relations measured before and during application of 5 nM IL-6 are shown in Fig. 2C. From a holding potential of $-80$ mV, $I_{Ba}$ was activated with a peak current amplitude at $+20$ mV. IL-6 did not alter the current-voltage relationship. These results suggest that 5 nM IL-6 facilitated $I_{Ba}$ in MC3T3-E1 cells.

3.3. IL-6 inhibited $I_{Ba}$
In addition to facilitation, IL-6-induced inhibition of $I_{Ba}$ could be observed. Fig. 3A and B shows that 50 nM IL-6 inhibited $I_{Ba}$ as shown with raw current traces obtained at $+10$ mV from $-80$ mV in the absence and presence of IL-6. $I_{Ba}$ was evoked every 5 s with a 100 ms depolarizing voltage step to $+10$ mV from a holding potential of $-80$ mV. As shown in Fig. 3A, application of 50 nM IL-6 inhibited $I_{Ba}$ from $-328$ pA to $-236$ pA (28.0% inhibition) in this cell.

To investigate the voltage dependency of facilitation of $I_{Ba}$ by 50 nM IL-6, we analyzed the current-voltage relations in the absence and presence of 50 nM IL-6. The current-voltage relations measured before and during application of 50 nM IL-6 are shown in Fig. 3C. From a holding potential of $-80$ mV, $I_{Ba}$ was activated with a peak current amplitude at $+20$ mV. IL-6 did not alter the current-voltage relationship. These results suggest that 50 nM IL-6 inhibited $I_{Ba}$ in MC3T3-E1 cells.

3.4. IL-1β and -6 modulated $I_{Ba}$ depends on concentration

The concentration-response relationship in the IL-1β and -6-induced modulation of $I_{Ba}$ is shown in Fig. 4A and B, respectively.

Application of 50 pM-50 nM IL-1β facilitated $I_{Ba}$. To generate a concentration-response curve, IL concentrations were applied randomly, and each MC3T3-E1 cells were exposed to only a single concentration. Fig. 4A shows that progressive voltage-dependent facilitations in IL-1β concentrations resulted in a progressively greater facilitation of $I_{Ba}$.

In addition, application of 50 pM-5 nM IL-6 facilitated $I_{Ba}$. In contrast to above results, 50 nM IL-6 inhibited $I_{Ba}$ in MC3T3-E1 cells.
4. Discussion

The present study investigated the effects of IL-1β and IL-6 on $I_{Ba}$ in MC3T3-E1 cells. Application of 50 pM-50 nM IL-1β facilitated $I_{Ba}$. Application of 50 pM-5 nM IL-6 facilitated $I_{Ba}$. In contrast, 50 nM IL-6 inhibited $I_{Ba}$ in MC3T3-E1 cells.

In osteoblastic cells, several studies demonstrated that hormones modulated VSCCs, included in parathyroid hormone (PTH) [20-22], bradykinin [23] and 1,25-dihydroxyvitamin D$_3$ (1,25(OH)$_2$D$_3$) [24-26]. To our knowledge, the data presented here demonstrate for the first time that IL-1β and IL-6 modulates VSCCs in osteoblasts. In contrast to our results, Green et al. reported that IL-6 did not alter basal intracellular $Ca^{2+}$ concentration ([Ca$^{2+}$]$_i$) but inhibited $Ca^{2+}$ transient induced by PTH, prostaglandin E$_2$ and endothelin-1 in osteoblast [27].

In this study, we have shown that 50 pM-50 nM IL-1β facilitated $I_{Ba}$. It has been demonstrated that local treatment with IL-1β (0.5 ng/h) for 72 h resulted in increased numbers of osteoblasts 14 days as measured histologically [28]. Increased DNA synthesis in response to IL-1β in vitro has been observed in osteoblast [29] and especially MC3T3-E1 cells [30]. In contrast, however, it has been reported that IL-1β inhibits the stimulation of osteocalcin synthesis and has no influence on proliferation [31]. IL-1β also increases both osteoprotegerin protein release and mRNA levels [32,33]. Recent study demonstrated that treatment with IL-1β resulted in biphasic effects on osteoblast differentiation. Short-term exposure (2 days) to IL-1β early in culture induces differentiation. Longer term exposure (6 days) to IL-1β inhibits osteoblast differentiation [34].
In this study, we have shown that 50 pM-5 nM IL-6 facilitated I_{Ba}. In contrast, 50 nM IL-6 inhibited I_{Ba}. What is the physiological relevance of IL-6-induced dual modulation of VSCCs, i.e. both facilitation and inhibition? Several studies indicated that IL-6 enhance in vitro differentiation on osteoblasts [35-40]. In contrast to above reports, other studies have shown an inhibitory effect of IL-6 on bone formation [41,42]. It can be considered that such dual effects are depends on IL-6 receptor's intracellular signals transduction mechanisms. For example, activation of STAT3 is necessary for osteoblast differentiation and bone formation induced by IL-6 [39]. On the other hand, PKCδ and ERK1/2 are implicated in IL-6's inhibitory effect on bone formation [43]. It is also possible that these dual effects depend on the differentiation stage of the osteoblast. IL-6 would stimulate the first stages of differentiation but on more mature cells, they would prevent further stimulation [41,42].

In this study, we used 50 pM-50 nM IL-1β and IL-6 to modulate I_{Ba}. These IL concentrations are quite high. In normal state, serum IL-1β concentration is 0.9 pg/ml approximately [44]. In the electrophysiological study and the immunocytochemistry study, however, several study demonstrated that high concentration, i.e. 0.57 nM and 4.5 nM, of IL was used to modulate ion channel activity and protein expression in single neuron [45,46]. IL-1β and -6 were applied by perfusion pressure ejection from perfusion tubes with a tip diameter of 1 mm placed within 10 mm of the cell surface. Although the concentration of IL-1β and -6 actually seen by the cell is certainly lower than that contained in the stock solution, we applied the IL-1β and -6 using same perfusion system.

We also observed that I_{Ba} were recovered to values of peak I_{Ba} after IL wash out using time course graph (Figs. 1B, 2B and 3B). Therefore, it can be considered that 50 pM-50
nM IL-1β and IL-6 had no effect on the cell function.

In this study, we used mouse MC3T3-E1 cell line. Chung et al. showed significant changes in MC3T3-E1 cell morphology, cell proliferation, osteoblastic function, and responsiveness to various protein after multiple passage (> 65) [47]. In a further study, cell passage numbers should be defined to prevent change the cell function.

As mentioned above, VSCCs are essential in the regulation of many cellular processes including gene expression and cell growth. Thus, IL-1β and -6 modulation of somatic Ca^{2+} influx via VSCCs could potentially affect Ca^{2+}-dependent gene expression and cell development [11, 12, 14].

5. Conclusion

In conclusion, IL-1β facilitated VSCCs in MC3T3-E1 cells. In addition, we acquired two different groups of results in IL-6: IL-6-induced facilitation and inhibition of VSCCs in MC3T3-E1 cells.
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Figure legends
Fig. 1. (A) Typical superimposed $I_{Ba}$ traces according to the time course graph B. $I_{Ba}$ was evoked from a holding potential of $-80$ mV by a 100 msec voltage step to $-10$ mV at 5 sec intervals. (B) Typical time course of 5 nM IL-1β-induced facilitation of $I_{Ba}$. IL-1β (5 nM) was bath-applied during the times indicated by the filled bar. (C) Current-voltage relationship of $I_{Ba}$ evoked by a series of voltage steps from a holding potential of $-80$ mV to test potentials between $-40$ and $+90$ mV in $+10$ mV increments in the absence (opened circles) and presence (filled circles) of 5 nM IL-1β.
Fig. 2. (A) Typical superimposed $I_{Ba}$ traces according to the time course graph B. $I_{Ba}$ was evoked from a holding potential of $-80 \text{ mV}$ by a 100 msec voltage step to $-10 \text{ mV}$ at 5 sec intervals. (B) Typical time course of 5 nM IL-6-induced facilitation of $I_{Ba}$. IL-6 (5 nM) was bath-applied during the times indicated by the filled bar. (C) Current-voltage relationship of $I_{Ba}$ evoked by a series of voltage steps from a holding potential of $-80 \text{ mV}$ to test potentials between $-40$ and $+90 \text{ mV}$ in $+10 \text{ mV}$ increments in the absence (opened circles) and presence (filled circles) of 5 nM IL-6.
Fig. 3. (A) Typical superimposed $I_{Ba}$ traces according to the time course graph B. $I_{Ba}$ was evoked from a holding potential of $-80$ mV by a 100 msec voltage step to $-10$ mV at 5 sec intervals. (B) Typical time course of 50 nM IL-6-induced inhibition of $I_{Ba}$. IL-6 (50 nM) was bath-applied during the times indicated by the filled bar. (C) Current-voltage relationship of $I_{Ba}$ evoked by a series of voltage steps from a holding potential of $-80$ mV to test potentials between $-40$ and $+90$ mV in $+10$ mV increments in the absence (opened circles) and presence (filled circles) of 50 nM IL-6.
Fig. 4. (A) Histogram demonstrating the degree of $I_{Ba}$ modulation by 50 pM-50 nM IL-1β.

(B) Histogram demonstrating the degree of $I_{Ba}$ modulation by 50 pM-50 nM IL-6.