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Effect of celecoxib on Ca^{2+} movement and cell proliferation in human osteoblasts

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Abstract

In human osteoblasts, the effect of the widely prescribed cyclooxygenase-2 inhibitor celecoxib on intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) and cell proliferation was explored by using fura-2 and the tetrazolium assay, respectively. Celecoxib at concentrations greater than 1 μM caused a rapid rise in $[Ca^{2+}]_i$ in a concentration-dependent manner (EC_{50} = 10 μM). Celecoxib-induced $[Ca^{2+}]_i$ rise was reduced by 90% by removal of extracellular Ca^{2+}, and by 30% by L-type Ca^{2+} channel blockers. Celecoxib-induced Mn^{2+}-associated quench of intracellular fura-2 fluorescence also suggests that celecoxib-induced extracellular Ca^{2+} influx. In Ca^{2+}-free medium, thapsigargin, an inhibitor of the endoplasmic reticulum Ca^{2+}-ATPase, caused a monophasic $[Ca^{2+}]_i$ rise, after which the increasing effect of celecoxib on $[Ca^{2+}]_i$ was greatly inhibited. Conversely, pretreatment with celecoxib to deplete intracellular Ca^{2+} stores totally prevented thapsigargin from releasing more Ca^{2+}. U73122, an inhibitor of phoispholipase C, abolished histamine (an inositol 1,4,5-trisphosphate-dependent Ca^{2+} mobilizer)-induced, but not celecoxib-induced, $[Ca^{2+}]_i$ rise. Pretreatment with phorbol 12-myristate 13-acetate and forskolin to activate protein kinase C and adenylate cyclase, respectively, partly inhibited celecoxib-induced $[Ca^{2+}]_i$ rise in Ca^{2+}-containing medium. Separately, overnight treatment with 1–100 μM celecoxib inhibited cell proliferation in a concentration-dependent manner. These findings suggest that in human osteoblasts, celecoxib increases $[Ca^{2+}]_i$ by stimulating extracellular Ca^{2+} influx and also by causing intracellular Ca^{2+} release from the endoplasmic reticulum via a phospholiase C-independent manner. Celecoxib may be cytotoxic at higher concentrations.

Keywords: Ca^{2+}; Ca^{2+} stores; Celecoxib; Fura-2; MG63; Osteoblasts

1. Introduction

Cyclooxygenase-2 (COX-2) is an important cellular target for both therapy and/or prevention of inflammatory disorders and cancer. The advent of selective COX-2 inhibitors now allows a more precise and safer treatment approach [1–3]. Because of their better gastrointestinal risk profile, the newly developed selective COX-2 inhibitors celecoxib and rofecoxib are discussed as cost-effective alternatives to common NSAIDs [1]. Due to the wide prescription of celecoxib in treating bone-related disorders, understanding the *in vitro* effect of celecoxib on osteoblasts is crucial. However, this issue has not been addressed before except that the prevention of prostaglandin synthesis by inflammatory cytokines in bone cells was thought to contribute to the efficacy of celecoxib in preventing bone loss in rheumatoid arthritis [3].

A regulated rise in cytosolic free Ca^{2+} levels ($[Ca^{2+}]_i$) is a key signal in all cell types, and can trigger many physiopathological events [4–6]; but an unregulated elevation in $[Ca^{2+}]_i$ is often cytotoxic [7]. Thus, it is important to examine the effect of an agent on cellular Ca^{2+} signaling in order to understand its *in vitro* effect. The effect of celecoxib on $[Ca^{2+}]_i$ in osteoblasts is unclear. However, in prostate cancer cells (PC3), it was shown that exposure of PC3 cells to celecoxib stimulates an immediate $[Ca^{2+}]_i$ rise in a dose- and time-dependent manner. This activity is highly specific for celecoxib, and is not noted with other

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COX inhibitors, including aspirin, ibuprofen, naproxen, rofecoxib, DuP697 and NS398 [8].

In the present study, MG63 human osteoblasts were used to investigate the effect of celecoxib on $[Ca^{2+}]_i$ in osteoblasts. MG63 cells have properties similar to human osteoblasts and have been widely used as a system for investigation of osteoblasts [9]. Many endogenous and exogenous agents can stimulate MG63 cells by causing a $[Ca^{2+}]_i$ increase, such as 2,2′-dithiodipyridine [10], riluzole [11], and tamoxifen [12]. The inositol 1,4,5-trisphosphate-sensitive Ca^{2+} store is an important Ca^{2+} store that releases Ca^{2+} into the cytosol when cells are stimulated by endogenous agents such as histamine [13]. But exogenous agents can release Ca^{2+} from inositol 1,4,5-trisphosphate-insensitive stores [10–12]. Like other non-excitable cells, the Ca^{2+} release may induce Ca^{2+} influx across the plasma membrane via the process of store-operated Ca^{2+} entry [14].

Using fura-2 as a fluorescent Ca^{2+} indicator, this study shows that celecoxib induced a significant $[Ca^{2+}]_i$ rise in a concentration-dependent manner in MG63 cells. The time course and the concentration–response relationship, the Ca^{2+} sources of the Ca^{2+} signal, the role of phospholipase C in the signal have been explored. The effect of celecoxib on cell proliferation has also been examined using the tetrazolium assay.

2. Materials and methods

2.1. Cell culture

MG63 human osteoblasts were obtained from American Type Culture Collection and were cultured in Dulbecco's modified Eagle medium supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin. Cells were kept at 37° in 5% CO_2-containing humidified air.

2.2. Solutions

Ca^{2+}-containing medium (pH 7.4) had (in mM): NaCl 140; KCl 5; $MgCl_2$ 1; $CaCl_2$ 2; Hepes 10; glucose 5. Ca^{2+}-free medium contained similar components as Ca^{2+}-containing medium except that $CaCl_2$ was substituted with 0.1 mM EGTA. Agents were dissolved in water, ethanol or dimethyl superoxide as stock solutions. Final concentrations of organic solvents in the $[Ca^{2+}]_i$ measurements were less than 0.1% and did not alter basal $[Ca^{2+}]_i$.

2.3. $[Ca^{2+}]_i$ measurements

Trypsinized cells (10^6/mL) were allowed to recover in culture medium for 1 hr before being loaded with 2 μM fura-2/acetoxy methyl (fura-2/AM) for 30 min at 25°. The cells were washed and re-suspended in Ca^{2+}-containing medium. Fura-2 fluorescence measurements were performed in a water-jacketed cuvette (25°) with continuous stirring; the cuvette contained 1 mL of medium and 0.5 million cells. Fluorescence was monitored with a Shimadzu RF-5301PC spectrofluorophotometer by recording excitation signals at 340 and 380 nm and emission signal at 510 nm at 1-s intervals. Maximum and minimum fluorescence values were obtained by adding 0.1% Triton X-100 and 10 mM EGTA sequentially at the end of each experiment. $[Ca^{2+}]_i$ was calculated as described previously assuming a K_d of 155 nM [15]. Mn^{2+} quench of fura-2 fluorescence was performed in Ca^{2+}-containing medium containing 50 μM $MnCl_2$, by recording the Ca^{2+}-insensitive excitation signal at 360 nm (emission signal at 510 nm) at 1-s intervals.

2.4. Cell proliferation assay

The measurement of cell proliferation is based on the ability of viable cells to cleave tetrazolium salts by mitochondrial dehydrogenase. Augmentation in the amount of developed color directly correlates with the number of metabolically active cells. Assays were performed according to manufacturer instructions (Roche Molecular Biochemical). Briefly, cells are seeded in 96-well plates at a density of 10,000 cells per well in culture medium for 16 hr to allow attachment. The next day the culture medium was replaced with 100 μL of serum-free medium containing different concentrations of celecoxib. The cell proliferation reagent WST-1 4-[3-[4-Iodophenyl]-2-4(4-nitrophenyl)-2*H*-5-tetrazolio-1,3-benzene disulfonate] (10 μL pure solution) was added to each sample 16 hr after celecoxib treatment, and cells were incubated for additional 30 min in a humidified atmosphere (37°). The absorbance of samples (A_{450}) was determined using a scanning multiwell spectrophotometer, that is enzyme-linked immunosorbent assay (ELISA) reader. Absolute optical density was normalized to the absorbance of unstimulated cells in each plate and expressed as a percentage of the control value. Experiments were repeated five times in six replicates (wells).

2.5. Chemicals

The reagents for cell culture were from Gibco (Gaithersburg). Fura-2/AM was from Molecular Probes. U73122 (1-(6-((17β-3-methoxyestra-1,3,5(10)-trien-17-yl)amino)-hexyl)-1*H*-pyrrole-2,5-dione) and U73343 (1-(6-((17β-3-methoxyestra-1,3,5(10)-trien-17-yl)amino)hexyl)-2,5-pyrrolidine-dione) were from Biomol (Plymouth Meeting). Celecoxib was a gift from Pfizer. The other reagents were from Sigma.

2.6. Statistics

Statistical comparisons were determined by using Student's *t*-test, and significance was accepted when $P < 0.05$.

3. Results

In Ca^{2+}-containing medium, the basal $[Ca^{2+}]_i$ was 50 ± 2 nM (N = 5). Addition of celecoxib (>0.1 μM) caused an immediate rise in $[Ca^{2+}]_i$, which lasted for, at least, 200 s after the addition of celecoxib (Fig. 1A); e.g. celecoxib (50 μM)-induced $[Ca^{2+}]_i$ rise attained to 451 ± 3 nM (N = 5; trace a) over the baseline. The Ca^{2+} signal was followed by a gradual decay that reached a level of 301 ± 2 nM over the baseline at the time point of 250 s. The increasing effect of celecoxib was concentration-dependent with an EC_{50} of 10 μM (Fig. 1C; filled circles).

To examine whether/how influx of extracellular Ca^{2+} and/or mobilization of Ca^{2+} from the intracellular store site(s) may contribute to celecoxib-induced $[Ca^{2+}]_i$ rise, the effect of celecoxib on $[Ca^{2+}]_i$ was measured in the absence of extracellular Ca^{2+}. Figure 1B shows that the $[Ca^{2+}]_i$ rise caused by 50 μM celecoxib was attenuated, with no change in the basal $[Ca^{2+}]_i$ (51 ± 1 nM, N = 5). Celecoxib increased $[Ca^{2+}]_i$ by 146 ± 2 nM at the time point of 50 s. The net area under the curve (during the 200 s after addition of celecoxib) of the celecoxib-induced responses was smaller by $92 \pm 2\%$ ($P < 0.05$) than that observed in Ca^{2+}-containing medium. At the time point of 250 s, 3 mM Ca^{2+} was added to induce extracellular Ca^{2+} influx. After pretreatment with celecoxib, addition of Ca^{2+} caused an immediate $[Ca^{2+}]_i$ rise with a value of 201 ± 3 nM, which was greater than control (without celecoxib pretreatment; 24 ± 2 nM) by 8.3 folds ($P < 0.05$; N = 5). These data suggest that celecoxib induced both extracellular Ca^{2+} influx and intracellular Ca^{2+} release, with the former playing a dominant role. The concentration–response curves of celecoxib-induced $[Ca^{2+}]_i$ rises in Ca^{2+}-containing medium and in Ca^{2+}-free medium are shown in Fig. 1C. The two curves in Fig. 1C suggest that Ca^{2+} influx contributed to about 90% of 1–100 μM celecoxib-induced $[Ca^{2+}]_i$ rise.

Further experiments were performed to exclude the possibility that the smaller celecoxib-induced response in Ca^{2+}-free medium was caused by EGTA-induced depletion of intracellular Ca^{2+}. Mn^{2+} enters cells through similar pathways as Ca^{2+} but quenches fura-2 fluorescence at all excitation wavelengths [16]. Thus, quench of fura-2 fluorescence excited at the Ca^{2+}-insensitive excitation wavelength of 360 nm by Mn^{2+} indicates Ca^{2+} influx. Figure 2 shows that 50 μM celecoxib induced an immediate decrease in the 360 nm excitation signal (compared to control; N = 5; $P < 0.05$). The maximal decrease occurred at the time point of 80 s with a value of 95 ± 2 nM (N = 5). This suggests that celecoxib-induced $[Ca^{2+}]_i$ rise involved Ca^{2+} influx from extracellular space.

Several L-type Ca^{2+} channel blockers were used to distinguish the Ca^{2+} entry pathways of celecoxib-induced $[Ca^{2+}]_i$ rise. Figure 3 shows that the $[Ca^{2+}]_i$ rise was inhibited by about 30% by 10 μM of these blockers (N = 5; $P < 0.05$).

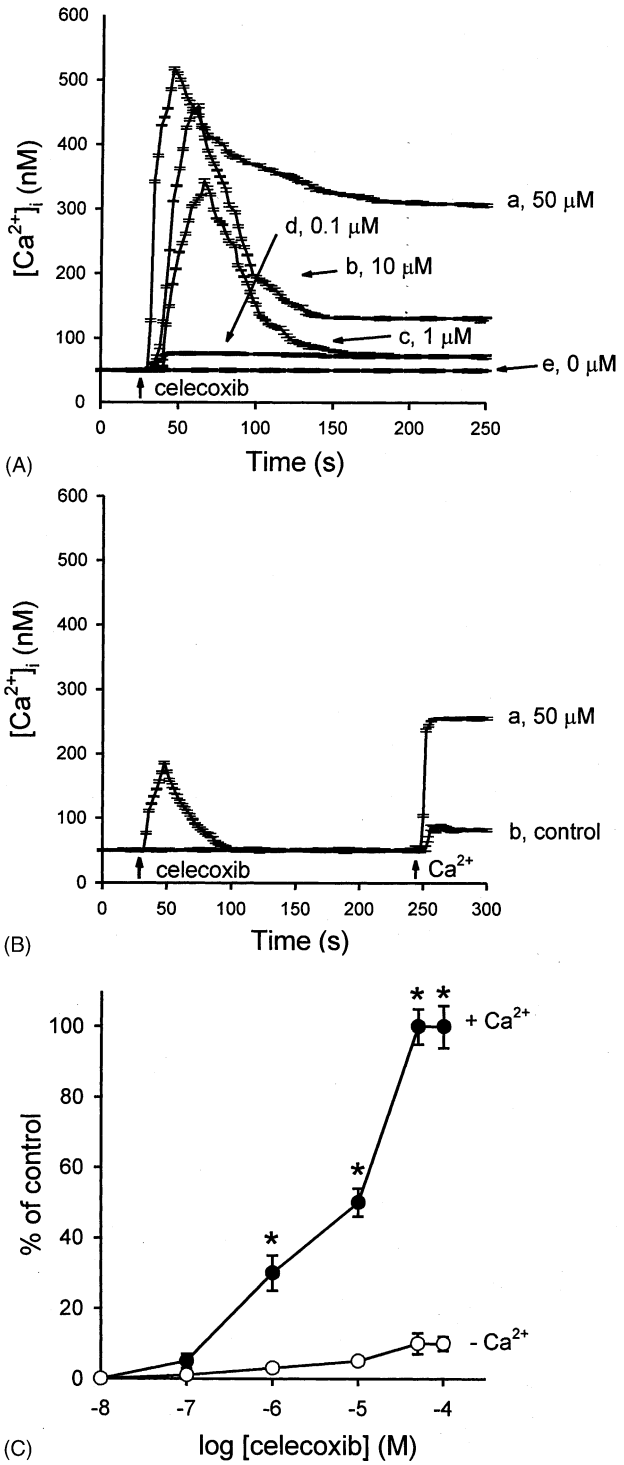

Fig. 1. Celecoxib-induced concentration-dependent $[Ca^{2+}]_i$ rises in MG63 osteoblasts. (A) In Ca^{2+}-containing medium, celecoxib was added at 25 s. The concentration of celecoxib was indicated by arrows. (B) Effect of removal of extracellular Ca^{2+} on celecoxib-induced response. The experiments were performed in Ca^{2+}-free medium (no added Ca^{2+} plus 0.1 mM EGTA). The concentration of celecoxib was 50 μM in trace a and 0 μM in trace b. $CaCl_2$ (3 mM) was added at 250 s to cause extracellular Ca^{2+} influx. (C) The concentration–response plots of celecoxib-induced Ca^{2+} signals. The *y*-axis is the percentage of control. Control was the net (baseline subtracted) area under the curve between 25 and 250 s of 50 μM celecoxib-induced $[Ca^{2+}]_i$ rise. Data are means ± SEM of five experiments.

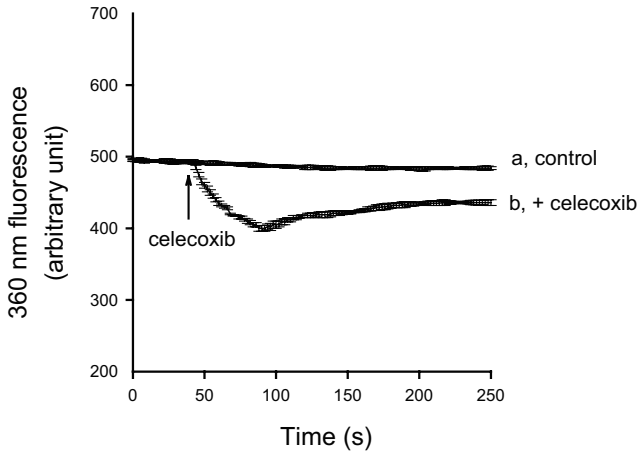

Fig. 2. Effect of celecoxib on Ca^{2+} influx by measuring Mn^{2+} quench of fura-2 fluorescence. Experiments were performed in Ca^{2+}-containing medium. $MnCl_2$ (50 μM) was added to cells before fluorescence measurements. The *y*-axis is fluorescence intensity (in arbitrary units) measured at the cinsensitive excitation wavelength of 360 nm and the emission wavelength of 510 nm. Trace a: no celecoxib was present. Trace b: 50 μM celecoxib was added at 45 s. Data are mean ± SEM of five experiments.

We examined whether celecoxib-induced $[Ca^{2+}]_i$ rise involves the mobilization of intracellular Ca^{2+} stored within the endoplasmic reticulum. Figure 4A shows that in Ca^{2+}-free medium, after treatment with 50 μM celecoxib for 450 s, addition of 1 μM thapsigargin, an inhibitor of the endoplasmic reticulum Ca^{2+}-ATPase [17], failed to release more Ca^{2+}. Conversely, Fig. 4B shows that addition of thapsigargin increased $[Ca^{2+}]_i$ by 75 ± 2 nM (N = 5) in a rapid monophasic manner. The signal returned to the basal level in 1 min. Addition of celecoxib at the time point of 500 s increased $[Ca^{2+}]_i$ by 19 ± 2 nM (N = 5), a value

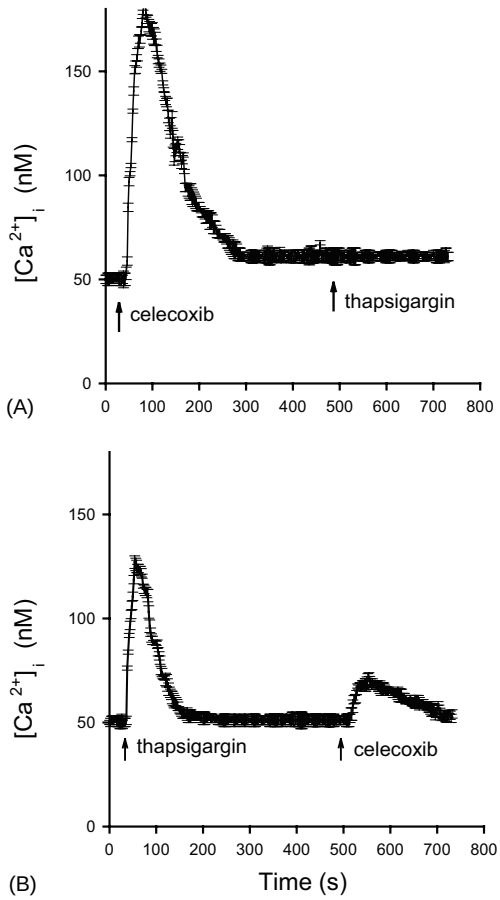

Fig. 4. Intracellular Ca^{2+} stores of celecoxib-induced $[Ca^{2+}]_i$ rise. The experiments were performed in Ca^{2+}-free medium. The agents were added at the time points indicated by arrows. The concentration of agents was 1 μM for thapsigargin and 50 μM for celecoxib. Data are means ± SEM of five experiments.

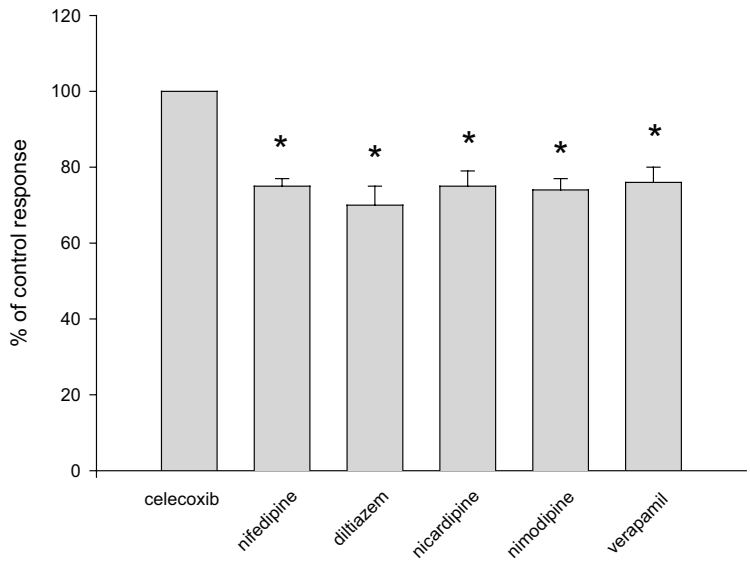

Fig. 3. Effect of L-type Ca^{2+} channels blockers on celecoxib-induced $[Ca^{2+}]_i$ rise. The control response is the $[Ca^{2+}]_i$ increase induced by 50 μM celecoxib in Ca^{2+}-containing medium. The blocker (10 μM) was added 1 min before celecoxib. Control was the net (baseline subtracted) area under the curve between 100 and 350 s of 50 μM celecoxib-induced $[Ca^{2+}]_i$ rise. Data are the mean ± SEM of five experiments. $^*P < 0.05$ compared with control.

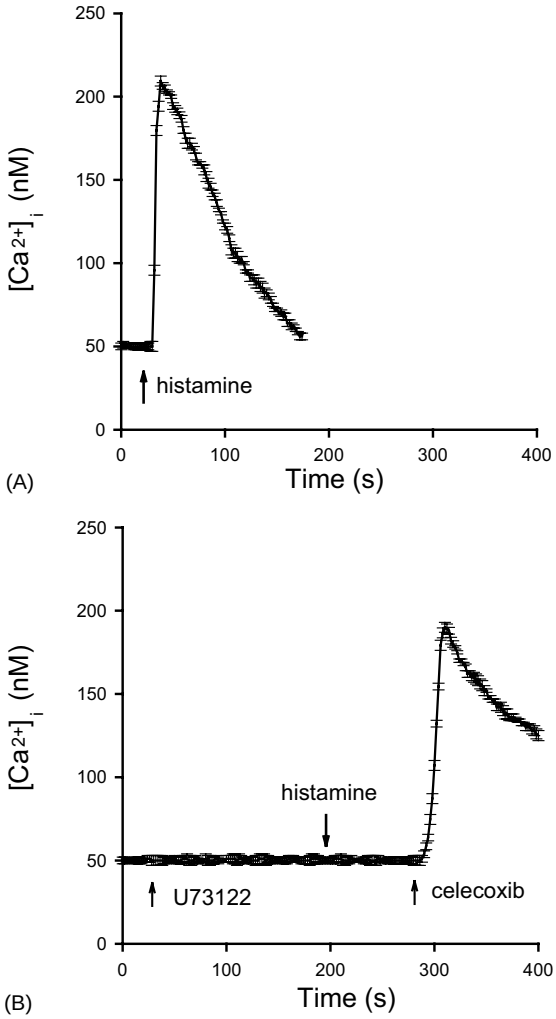

Fig. 5. Lack of involvement of phospholipase C in celecoxib-induced $[Ca^{2+}]_i$ rise. The experiments were performed in Ca^{2+}-free medium. (A) Histamine (10 μM) was added at 20 s. (B) U73122 (2 μM), histamine (10 μM) and celecoxib (50 μM) were added at the time points indicated by arrows. Data are mean ± SEM of five experiments.

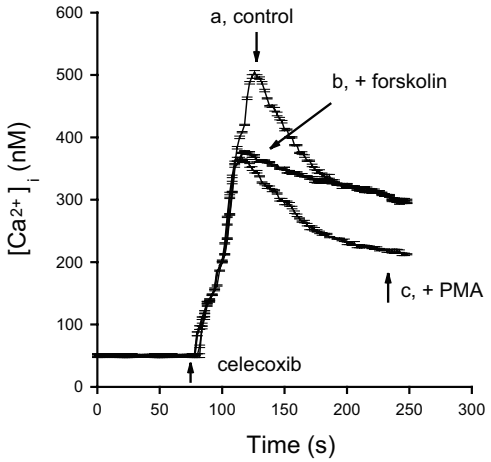

Fig. 6. The role of protein kinase C and cAMP in celecoxib-induced $[Ca^{2+}]_i$ rise. The experiments were performed in Ca^{2+}-containing medium. Phorbol 12-myristate 13-acetate (PMA; 1 nM) or forskolin (100 μM) was added at 20 s, and did not alter basal $[Ca^{2+}]_i$. Celecoxib (50 μM) was added at 75 s. Trace a: control: in the absence of PMA and forskolin. Trace b: with forskolin pretreatment. Trace c: with PMA pretreatment. Data are mean ± SEM of five experiments.

smaller than that shown in Fig. 4A (126 ± 3 nM) by 85% ($P < 0.05$; N = 5). This suggests that celecoxib releases intracellular Ca^{2+} mainly from the endoplasmic reticulum.

The possibility that phospholipase C-inositol 1,4,5-trisphosphate pathway is involved in celecoxib-induced Ca^{2+} release was examined. Figure 5A shows that 10 μM histamine, an agonist for H1 type histamine receptors that mobilizes intracellular Ca^{2+} via increasing inositol 1,4,5-trisphosphate in MG63 cells [13], caused an instantaneous monophasic $[Ca^{2+}]_i$ rise (152 ± 2 nM, N = 5) in Ca^{2+}-free medium. Figure 5B, however, shows that pretreatment with 2 μM U73122, an inhibitor of phospholipase C [18], abolished histamine-induced $[Ca^{2+}]_i$ rise; in contrast, 10 μM U73343, a biologically inactive analogue of U73122 [18], failed to prevent histamine-induced $[Ca^{2+}]_i$ rise (data not shown, N = 4). Even in the presence of 2 μM U73122, 50 μM celecoxib caused a significant $[Ca^{2+}]_i$ rise by 142 ± 2 nM (N = 5), a value indistinguishable from the celecoxib-induced $[Ca^{2+}]_i$ rise in control groups (Fig. 1B).

It has been shown that protein kinase C and cAMP may play a significant role in Ca^{2+} signaling in osteoblasts [19–21]. The data in Fig. 5 show that, in Ca^{2+}-containing medium, pretreatment with phorbol myristate acetate (PMA; 10 nM) to activate protein kinase C caused a significant decrease in 50 μM celecoxib-induced $[Ca^{2+}]_i$ rise. The net area under the curve was decreased by 24 ± 2% ($P < 0.05$; N = 5), and the net maximum value was decreased by 26 ± 2% (330 ± 3 nM vs. 451 ± 2 nM; $P < 0.05$; N = 6). Furthermore, pretreatment with 10 μM forskolin to increase cAMP levels resulted in a decrease in celecoxib-induced $[Ca^{2+}]_i$ rise by 17 ± 2% ($P < 0.05$) in the net area under the curve. The net maximum value was decreased by 26 ± 1% ($P < 0.05$).

It is well established that unregulated, prolonged $[Ca^{2+}]_i$ rises may lead to cytotoxicty [7], thus experiments were performed to examine the effect of overnight incubation with celecoxib on the proliferation of osteoblasts. Based on the tetrazolium assay, in control groups, cell number per well increased by 20.4% from 10,356 ± 121 cells/well before experiments to 13,024 ± 145 (N = 5; six replicates in each experiment; $P < 0.05$). Figures 6 and 7 show that in the presence of up to 10 μM celecoxib, the cell number did not change. However, in the presence of 40, 70 and 100 μM celecoxib, cell numbers significantly decreased by 18 ± 2%, 25 ± 10% and 42 ± 2%, respectively (N = 5; $P < 0.05$).

4. Discussion

The current study asked the question whether celecoxib could alter $[Ca^{2+}]_i$ and cell proliferation in human osteoblasts. In osteoblasts, it has been shown that a well-tuned

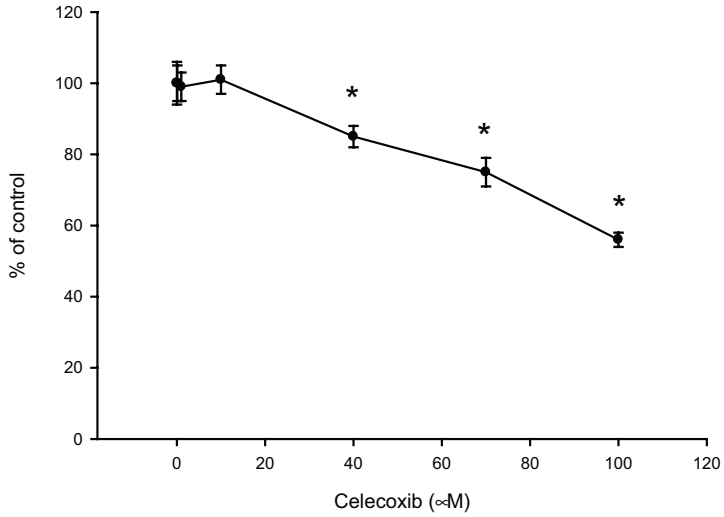

Fig. 7. A tetrazolium assay of the effect of celecoxib on proliferation of MG63 cells. Cells were treated with different concentrations of celecoxib for overnight, and an ELISA assay was performed as described in Section 2. Data are mean ± SEM of five experiments. Each treatment had six replicates (wells). Data are expressed as percentage of control that is the increase in cell number in celecoxib-free group. Control had 10,356 ± 121 cells/well before experiments, and had 13,024 ± 145 cells/well after incubation for overnight $^{*}P < 0.05$ compared to control.

$[Ca^{2+}]_i$ rise is important for normal cell function, such as release of ATP [22], but unregulated $[Ca^{2+}]_i$ rises are cytotoxic [7]. The data suggest that celecoxib evoked a concentration-dependent $[Ca^{2+}]_i$ rise with an EC_{50} of 10 μM. The Ca^{2+} signal was mainly contributed by extracellular Ca^{2+} influx and also by intracellular Ca^{2+} release because the signal was reduced by 90% by removing extracellular Ca^{2+}. This decrease in Ca^{2+} response was not caused by EGTA-induced depletion of Ca^{2+} stores, because the Mn^{2+} quench experiments suggest that celecoxib induced Ca^{2+} influx.

MG63 cells have been shown to possess Ca^{2+} stores in the endoplasmic reticulum, mitochondria and other unknown compartments [10–13]. Among these stores, celecoxib appears to mainly release Ca^{2+} from thapsigargin-sensitive endoplasmic reticulum Ca^{2+} stores because depletion of the stores with thapsigargin inhibited a major part of celecoxib-induced Ca^{2+} release, and conversely, pretreatment with celecoxib abolished thapsigargin-sensitive Ca^{2+} release. It is unlikely that phospholipase C was involved in celecoxib-induced Ca^{2+} release since the release was not changed by suppressing this enzyme. How celecoxib causes $[Ca^{2+}]_i$ increases in the absence of extracellular Ca^{2+} is unclear, but it may act in a manner similar to thapsigargin given the evidence that it can inhibit endoplasmic reticulum Ca^{2+} pump in human prostate cells [8].

Addition of L-type Ca^{2+} channel blockers inhibited celecoxib-induced $[Ca^{2+}]_i$ rise by 30%, suggesting that a part of the Ca^{2+} influx is via L-type Ca^{2+} channels. This is consistent with previous reports that osteoblasts contain this type of Ca^{2+} channels [23–26]. Since 60% of the celecoxib Ca^{2+} influx was not inhibited by the blockers [10 μM; sufficient to fully block L channels], the other parts of Ca^{2+} influx may be mediated by store-operated Ca^{2+} entry, a process triggered by depletion of Ca^{2+} stores [14]. This is possible as suggested by the data that addition of extracellular Ca^{2+} induces immediate Ca^{2+} influx after intracellular Ca^{2+}stores are depleted by celecoxib. But the same results would be obtained if celecoxib directly causes extracellular Ca^{2+} influx independently of depletion of Ca^{2+} stores. The possibility that the celecoxib-induced Ca^{2+} influx is via store-operated Ca^{2+} entry was difficult to explore due to the lack of selective pharmacological inhibitors for this pathway [27]. Recently, a Ca^{2+}-activated non-selective cation channel (TRPM4) has been cloned in excitable and non-excitable cells [28]. TRPM4 is activated following receptor-mediated Ca^{2+} mobilization, representing a regulatory mechanism that controls the magnitude of Ca^{2+} influx by modulating the membrane potential and, with it, the driving force for Ca^{2+} entry through other Ca^{2+}-permeable pathways. Thus, it remains possible that Ca^{2+} entry mechanisms other than depletion-activated channels may be important in Ca^{2+} influx in non-excitable cells.

It has been shown that protein kinase C activation is intimately cross-linked with Ca^{2+} signaling in osteoblasts. Intracellular Ca^{2+} and protein kinase C have been shown to mediate expression of receptor activator of nuclear factor-kappaB ligand and osteoprotegerin [29]. Prostaglandin D_2 induces interleukin-6 synthesis via Ca^{2+} mobilization that is regulated by protein kinase C [30]. Furthermore, the heavy metal lead was shown to induce a $[Ca^{2+}]_i$ rise which is mediated through activation of protein kinase C [31]. These lines of evidence prompted us to explore the role of protein kinase C in celecoxib-induced $[Ca^{2+}]_i$ rise in MG63 cells, and it was found that the Ca^{2+} signal was significantly reduced by activation of protein kinase C. Furthermore, in human HOBIT osteoblastic cells, increased levels of cAMP were shown to induce post-translational modifications (i.e. phosphorylations) of connexin43 and enhancement of gap

junction assembly, resulting in an increased junctional permeance to Lucifer yellow and to a positive modulation of intercellular Ca^{2+} waves [21]. A relationship between cAMP and $[Ca^{2+}]_i$ in osteoblasts is supported by our data showing that increasing cAMP levels can inhibit celecoxib-induced $[Ca^{2+}]_i$ rise. Together, our data and previous reports suggest that Ca^{2+} signaling, protein kinase C and cAMP are closely associated in osteoblasts.

Substantial evidence indicates that celecoxib displays anti-tumor effect by sensitizing cancer cells to apoptosis. Exposure of these cells to celecoxib induces characteristic features of apoptosis, including morphological changes, DNA laddering, and caspase-3 activation [32–35]. Our data show that overnight treatment with celecoxib did not affect cell proliferation at concentrations up to 10 μM. But higher concentrations of the agent inhibited cell proliferation in a concentration-dependent manner.

In bone cells of the osteoblast lineage, Ca^{2+} channels play fundamental roles in cellular responses to external stimuli including both mechanical forces and hormonal signals. They are also proposed to modulate paracrine signaling between bone-forming osteoblasts and bone-resorbing osteoclasts at local sites of bone remodeling [36]. A $[Ca^{2+}]_i$ rise in osteoblasts is associated with activation of intracellular signaling pathways that control cell behavior and phenotype, including patterns of gene expression [36]. The effect of celecoxib on $[Ca^{2+}]_i$ and cell proliferation may provide a plausible link with the reported toxicities of celecoxib such as increased side effects in long-term therapy against disorders such as rheumatoid arthritis [37].

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