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Activation of Maxi-K Channels by Parathyroid Hormone and Prostaglandin E_2 in Human Osteoblast Bone Cells

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Abstract. Patch clamp experiments were performed on two human osteosarcoma cell lines (MG-63 and SaOS-2 cells) that show an osteoblasticlike phenotype to identify and characterize the specific K channels present in these cells. In case of MG-63 cells, in the cell-attached patch configuration (CAP) no channel activity was observed in 2 mM Ca Ringer (control condition) at resting potential. In contrast, a maxi-K channel was observed in previously silent CAP upon addition of 50 nM parathyroid hormone (PTH), 5 nM prostaglandin E_2 (PGE_2) or 0.1 mM dibutyryl cAMP + 1 μM forskolin to the bath solution. However, maxi-K channels were present in excised patches from both stimulated and nonstimulated cells in 50% of total patches tested. A similar K channel was also observed in SaOS-2 cells. Characterization of this maxi-K channel showed that in symmetrical solutions (140 mM K) the channel has a conductance of 246 ± 4.5 pS (n = 7 patches) and, when Na was added to the bath solution, the permeability ratio (P_K/P_{Na}) was 10 and 11 for MG-63 and SaOS-2 cells respectively. In excised patches from MG-63 cells, the channel open probability (P_o) is both voltage- (channel opening with depolarization) and Ca-dependent; the presence of Ca shifts the P_o *vs.* voltage curve toward negative membrane potential. Direct modulation of this maxi-K channel via protein kinase A (PKA) is very unlikely since in excised patches the activity of this channel is not sensitive to the addition of 1 mM ATP + 20 U/ml catalytic subunit of PKA. We next evaluated the possibility that PGE_2 or PTH stimulated the channel through a rise in intracellular calcium. First, calcium uptake ($^{45}Ca^{++}$) by MG-63 cells was stimulated in the presence of PTH and PGE_2, an effect inhibited by Nitrendipine (10 μM). Second, whereas PGE_2 stimulated the calcium-activated maxi-K channel in 2 mM Ca Ringer in 60% of patches studied, in Ca-free Ringer bath solution, PGE_2 did not open any channels (n = 10 patches) nor did cAMP + forskolin (n = 3 patches), although K channels were present under the patch upon excision. In addition, in the presence of 2 mM Ca Ringer and 10 μM Nitrendipine in CAP configuration, PGE_2 (n = 5 patches) and cAMP + forskolin (n = 2 patches) failed to open K channels present under the patch. As channel activation by phosphorylation with the catalytic subunit of PKA was not observed, and Nitrendipine addition to the bath or the absence of calcium prevented the opening of this channel, it is concluded that activation of this channel by PTH, PGE_2 or dibutyryl cAMP + forskolin is due to an increase in intracellular calcium concentration via Ca influx.

Key words: Patch clamp — Calcium activated K channel — Osteoblast — PTH — PGE_2

Introduction

Bone is a dynamic organ that undergoes continuous renewal. Under normal conditions bone resorption by osteoclasts is finely balanced to the amount of bone that is made by osteoblasts, such that total bone mass is conserved. In a pathological situation like osteoporosis, an imbalance between these two processes results in net bone loss. The mechanisms that underlie this condition are still debated, but may stem from disorders of cell-to-cell communication or signal transmission. One of the most important hormones in the control of bone resorption is parathyroid hormone (PTH). Although osteoclast cells resorb the bone, it was found that the receptors for PTH were located exclusively on the bone-forming osteoblast cells [44]. Thus, it was hypothesized that osteo-

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blast cells are the main targets for PTH and that they regulate osteoclastic activity, and hence resorption, via cell-to-cell communication. Likely candidates for second messengers to transmit the PTH response within a cell include cyclic AMP [27, 28, 30, 48] and intracellular calcium [30, 40, 48, 51], since PTH has been shown to cause a rise in both of these messengers in a variety of osteoblast cells.

PTH has been shown to cause multiphasic changes in the cell membrane potential resulting in a sustained hyperpolarization [10, 12, 13]. These changes in membrane potential possibly involve modification of currents through ionic channels. However, the ionic basis for these changes in membrane potential is not clear. There is indirect evidence for the existence of calcium-activated K channels in osteoblast cell models [8, 10], and embryonic chick osteoblast cell cultures [39, 53].

Other agents that cause bone resorption include prostaglandins [7, 26], the most potent prostaglandin, in terms of bone resorption, being PGE_2 [7]. It is believed that prostaglandins act as paracrine rather than circulating hormones, and many circulating hormones [46, 52], including PTH [33], have been found to stimulate the production and secretion of prostaglandins by osteoblasts. Prostaglandins have been shown to increase intracellular cAMP content in osteoblast cells [9, 49, 50, 54] and indeed in the human MG-63 osteosarcoma cell line intracellular cAMP is increased by PGE_2 [27]. The effect of prostaglandins on intracellular calcium content is less well studied, however, they appear to cause a rise in intracellular calcium concentration [49, 50].

The aims of this study were, first to directly identify by patch clamp recordings the potassium channels present in the human osteosarcoma cell lines MG-63 and SaOS-2, that display osteoblastlike properties [27, 28, 41], and second to see if PTH and PGE_2 have any effects on these channels. Our results indicate the presence of a calcium-activated, maxi-K channel in these cells. This channel is activated from otherwise silent patches by PTH and PGE_2. This effect is mimicked by cAMP + forskolin, yet it is not the direct target of protein kinase A phosphorylation. Indeed, our data suggest that PTH and PGE_2-dependent calcium influx into these cells is necessary for the activation of this channel. A possible role of this channel in the mediation of PTH and PGE_2 action in bone cells is discussed.

Materials and Methods

Cell Culture

Two human osteosarcoma cell lines (MG-63 and SaOS-2) presenting an osteoblastlike phenotype were purchased from the American Type Culture Collection (Rockville, Maryland). MG-63 cells were grown on impermeable supports in a 1:1 mixture of Ham F12 and Dulbecco's modified Eagle media (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin, while SaOS-2 cells were grown in McCoy's medium (Sigma, St. Louis, MO) supplemented with 10% FBS. Cells were harvested by trypsin-EDTA treatment (0.025% trypsin, 0.05% EDTA in phosphate buffered saline, Gibco, NY) and passaged at a ratio of 1:9 once a week for MG-63 cells and a ratio of 1:4 for SaOS-2 cells. Cells were passaged no more than 12 times and experiments were performed on cells between the 4th and 10th passage. Forty-eight hours before an experiment, cells were harvested and laid on glass coverslips at a density of 10,000 cells/cm^2 and after 24 hr the medium was changed to one containing 2% FBS, to reduce cell growth and synchronize the cells at the same point in the cell cycle.

Patch Clamp Studies

The glass coverslips on which the cells adhered were transferred to a chamber on the stage of an inverted microscope for the patch clamp experiments [21]. Patch pipettes (resistance 5 to 10 M ohm when filled with 140 mM KCl and placed in control Ringer solution, *see* solutions) were fabricated from hematocrit glass tubing (Fisher, Ottawa, Ontario) using a vertical two-stage patch pipette puller (Narishige, Japan). Current records were amplified with a patch clamp amplifier (L/M-EPC7, List, F.R.G.) and stored on videotape, following pulse code modulation (Neurodata DR-384, New York, NY).

Channel current records were analyzed using pClamp (Axon Instruments, version 5.5.1). The data were filtered at 500 Hz using an 8-pole Bessel filter (Frequency devices, MA) and sampled at 1 KHz into computer memory (a 386 IBM-compatible PC) via a TL-1 DMA labmaster interface (Axon Instruments, Foster City, CA). Channel conductances were estimated by linear regression over the voltage range indicated in the results section. The applied transpatch voltage, V corresponds to Vbath − Vpipette, where Vbath is always equal to zero. In cell-attached patches the potential across the patch is equal to the applied potential, V, plus the cell membrane potential, whereas in excised patches the potential across the patch is directly the applied potential, V. The channel open probability (P_o) was determined using the equation described by Marunaka and Tohda [35].

Solutions

In all the experiments, the pipette solution contained (in mM): 140 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 glucose, 5 HEPES adjusted to pH 7.4 with KOH, and the cells were initially bathed in control Ringer solution (2 mM Ca Ringer) containing (in mM): 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 glucose, 5 HEPES adjusted to pH 7.4 with NaOH. Some experiments were performed in 0 mM Ca Ringer containing also 1.5 mM EGTA.

For cell-attached patch experiments (CAP) the effect of the following drugs was studied: 50 nM parathyroid hormone (human PTH 1-34, Peninsula, CA), 5 nM prostaglandin E_2 (PGE_2, Sigma, St. Louis, MO) and 0.1 mM dibutyryl cAMP + 1 μM forskolin (Sigma, St. Louis, MO). In some assays, 10 μM Nitrendipine was used in combination with PTH, PGE_2 or cAMP + forskolin. The drugs were dissolved in control Ringer solution just prior to use, and during the recordings the cells were continually superfused.

Excised patch experiments were performed in symmetrical K solutions where the bath solution was identical to that of the pipette (140 mM K) or in Ringer solution (5 mM K), or in a solution containing 70 mM K and 70 mM Na. The low calcium Ringer solutions contained 1.5 mM EGTA and the free calcium concentration was adjusted to 10 μM, 2 μM or 1 μM by the addition of 1.49, 1.39 and 1.3 mM total calcium. The free calcium concentration was calculated according to

Fabiato and Fabiato [11]. In some excised patch experiments, the following were added to the Ringer solution (in mM): 1 $BaCl_2$, 1 or 5 ATP, 1 ATP + 20 U/ml catalytic subunit of protein kinase A (Sigma, St. Louis, MO), and 10 μM Nitrendipine (Sigma, St. Louis, MO). Care was taken to adjust the pH of the solutions containing either EGTA or ATP to 7.4 with NaOH.

Calcium Uptake

Cells grown to 80% confluency in 35 mm petri dishes were incubated in a 1:1 mixture of Ham F12 and Dulbecco's modified Eagle media without serum for 10 min at 37°C, before 1 mM $^{45}CaCl_2$ and either 50 nM PTH or 5 nM PGE_2 were added to the medium either in the presence or absence of 10 μM Nitrendipine (Sigma, St. Louis, MO). After a 15-min incubation the cells were washed three times with ice-cold stop solution (150 mM KCl and 15 mM Hepes-Tris pH 7.4) containing 2 mM EGTA and 1 μM La^{3+}, solubilized in 1N NaOH for 1 hr at 4°C and counted on a LKB counter. In individual experiments, triplicate dishes were used per conditions tested, and the results pooled for each experiment.

Statistics

Groups of paired and unpaired data were analyzed using Student's *t*-test and are presented as mean ± SEM. Mean values were assumed to be significantly different from each other when the *P* value was less than 0.05.

Results

The rate of seal formation on cells from the human osteosarcoma cell line MG-63 with 140 mM K in the patch pipette and 2 mM Ca Ringer in the bath was very high (83%, $n = 496$ total patches). Within these experiments, a maxi-K channel was observed under the pipette patch in about 50% of cases ($n = 210$). When a maxi-K channel was observed in the excised configuration, these patches were used for the characterization of this channel (conductance, selectivity, calcium and barium sensitivity, modulation of channel activity by hormones, etc.), and the individual number of patches (n) used for each different analysis is indicated where appropriate.

In cell-attached patches (CAP) we did not observe any channel currents when the cells were perfused with 2 mM Ca Ringer. In rare cases, depolarization of the patch by approximately 100 mV lead to brief openings of a large conductance channel. However, upon addition of 50 nM PTH or 5 nM PGE_2 to the bath solution while recording from a silent cell-attached patch at Vp = 0 mV, a sustained activation of a large conductance channel was frequently observed (50 to 60% of total assays when a maxi-K channel was present under the patch after excision, Table 1). From Fig. 1 steady state single channel inward currents of 8.4 ± 0.9 pA ($n = 8$ patches) can be observed upon stimulation of the cells with either PTH or PGE_2. Assuming the cells have a negative membrane potential, it is likely that the observed inward currents are carried by potassium ions flowing from the pipette to the cell.

Table 1. Single channel open probability (P_o) measurements in the presence of PTH, PGE_2 and dibutyryl cAMP + forskolin

	Single channel open probability (P_o)		Response frequency + Drug
	Control	+Drug	
PTH	0	0.69 ± 0.13 (3)	N.D.*
PGE_2	0	0.62 ± 0.1 (3)	60% (7/12)
cAMP + F	0	0.74 ± 0.04 (5)	53% (9/17)

The numbers in parenthesis are the number of experiments performed to calculate the open probability and, for the response frequency, the number of experiments in which a maxi-K channel was observed per total experiments performed. *N.D.: not determined since only three experiments were performed.

In addition to channel activation in the presence of PTH or PGE_2, we also observed a stimulation of a channel when a cocktail of 0.1 mM dibutyryl cAMP + 1 μM forskolin (Fig. 1, Table 1; 53% of 17 patches) was added to the bath solution while recording from a silent cell-attached patch ($V_p = 0$ mV). This channel seemed to be similar to the channel activated with PTH and PGE_2. A sustained channel activation was observed after an average time delay of 57 ± 10 sec following the addition of PTH, PGE_2 or dibutyryl cAMP + forskolin, which is only slightly longer than the delay required to completely exchange the bath solution (approximately 30 sec). The *I/V* relations for the channel activated in CAP after activation with PTH, PGE_2 or dibutyryl cAMP + forskolin are strikingly similar (Fig. 2). The channel has an inward conductance of 156 pS (determined between −40 and 40 mV), and it appears that the channel also displays some degree of inward rectification at higher imposed voltages. The currents reverse at a pipette potential of −51 mV suggesting a high selectivity for potassium in the CAP configuration.

Characterization of the channel observed in stimulated cells was performed in excised patches. A single channel type was observed on excision of patches from both stimulated and nonstimulated MG-63 cells and it appeared that the channel characteristics were independent of whether or not the cell had been prestimulated, therefore the data were pooled.

Typical current records in symmetrical K solutions, and the current-voltage relations for this channel in solutions with different K concentrations are depicted in Fig. 3 for MG-63 cells. Table 2 shows the conductance and reversal potential of the channel in the different K solutions for both MG-63 and SaOS-2 cells. Conductance measurements were taken between −40 and +40 mV in symmetrical 140 mM K solutions, between −40 and 0 mV in 70 K and between −20 and +20 mV in 5 mM

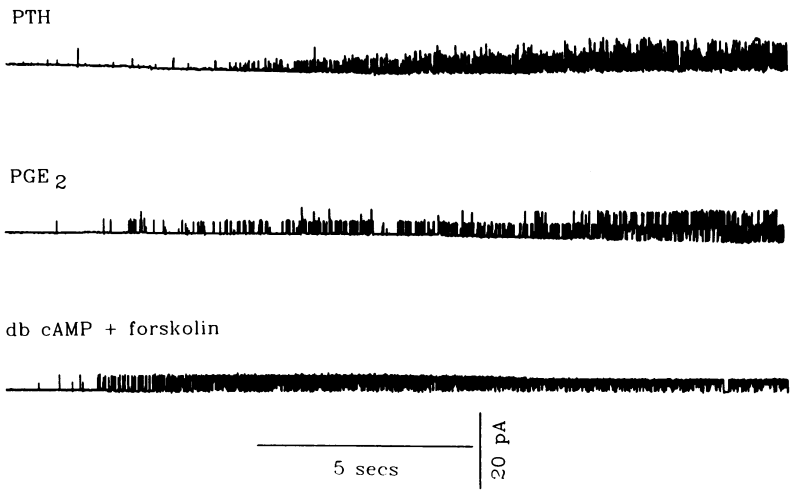

Fig. 1. Representative traces showing channel activation in cell-attached patches after the addition of; top: 50 nM PTH; middle: 5 nM PGE_2; bottom: 0.1 mM dibutyryl cAMP + 1 μM forskolin to the bathing solution. The recordings were taken between 40–80 sec after the addition of the drugs. The pipette solution was 140-mM buffered KCl solution and the solution bathing the cells was Ringer. Upward deflections represent current flow from the pipette to the cell.

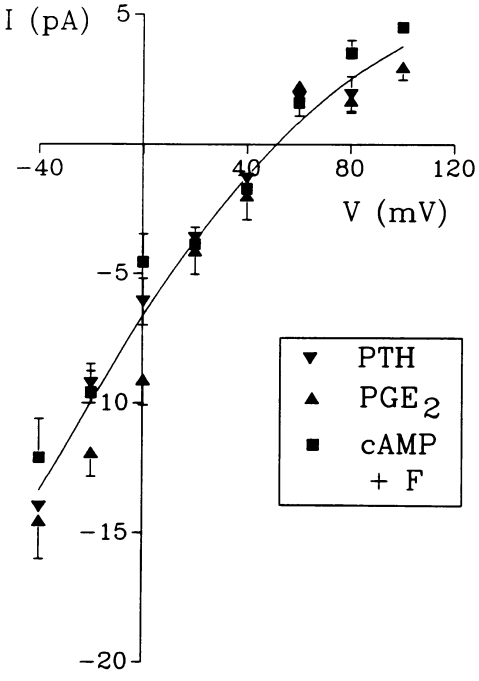

Fig. 2. Current-voltage relations from cell attached patches after stimulation of channel activity by PTH, PGE_2 or dibutyryl cAMP + forskolin. The solid line is a second order polynomial fit to the data. *V* is the applied voltage and corresponds to minus the potential applied to the pipette.

K. The K to Na selectivity of this channel was studied by replacing K by Na in the bathing solution with 140 mM K in the patch pipette. In symmetrical solutions the *I/V* relation is Ohmic and the reversal potential for the channel is, as expected for a K channel, around 0 mV. Lowering bath K concentration shifts the reversal potential towards E_K and the rectification displayed is due to the asymmetric ion concentration on either side of the patch, as predicted by Goldman, since both the *I/V* curves (where bath K was 5 or 70 mM) were fitted with the Goldman-Hodgkin-Katz equation using a P_K:P_{Na} ratio of 11 and 10 for MG-63 and SaOS-2 cells respectively. Thus, this channel is clearly selective for K over Na in both cell lines.

In excised patches the channel is exquisitely sensitive to the calcium concentration bathing the cytosolic face of the patch (Fig. 4). In 2 mM calcium, between transpatch potentials of −40 and 40 mV the channel is open for around 70% of the time and as seen from Fig. 3 (left) over this voltage range the channel open probability is not voltage-dependent under symmetrical K concentration conditions, whereas when the bath K concentration is lower, the current decreases at V_p higher than 40 mV. Lowering bath calcium concentration to 10 μM reduced slightly the P_o over the same voltage range. However, with 1 μM calcium bathing the intracellular face of the patch the channel was completely closed at negative pipette potentials, yet, P_o could be increased by large depolarizations of the patch of up to +80 mV as shown in Fig. 4.

The channel was blocked in a voltage-dependent manner by addition of 1 mM barium to the bath solution in excised patches. From Fig. 5 it can be seen that at positive pipette potentials ($V = -V_p$) where one might expect external barium to be repelled from the channel, P_o is only slightly reduced. However, the barium block is complete at negative pipette potentials where the positively charged barium would be expected to be drawn into the channel.

To test whether the activation of this channel in cell-attached patches was via an increase in intracellular cAMP leading to activation of protein kinase A (PKA) and phosphorylation of the channel, we performed experiments in excised patches where 1 mM ATP and 20 U/ml catalytic subunit of PKA were added to the bath Ringer which contained 2 mM calcium. The P_o under control conditions was 0.63 + 0.09 ($n = 4$ patches) and addition of catalytic subunit of PKA + 1 mM ATP did not significantly change P_o (0.74 ± 0.09, $n = 4$ patches, $P >$

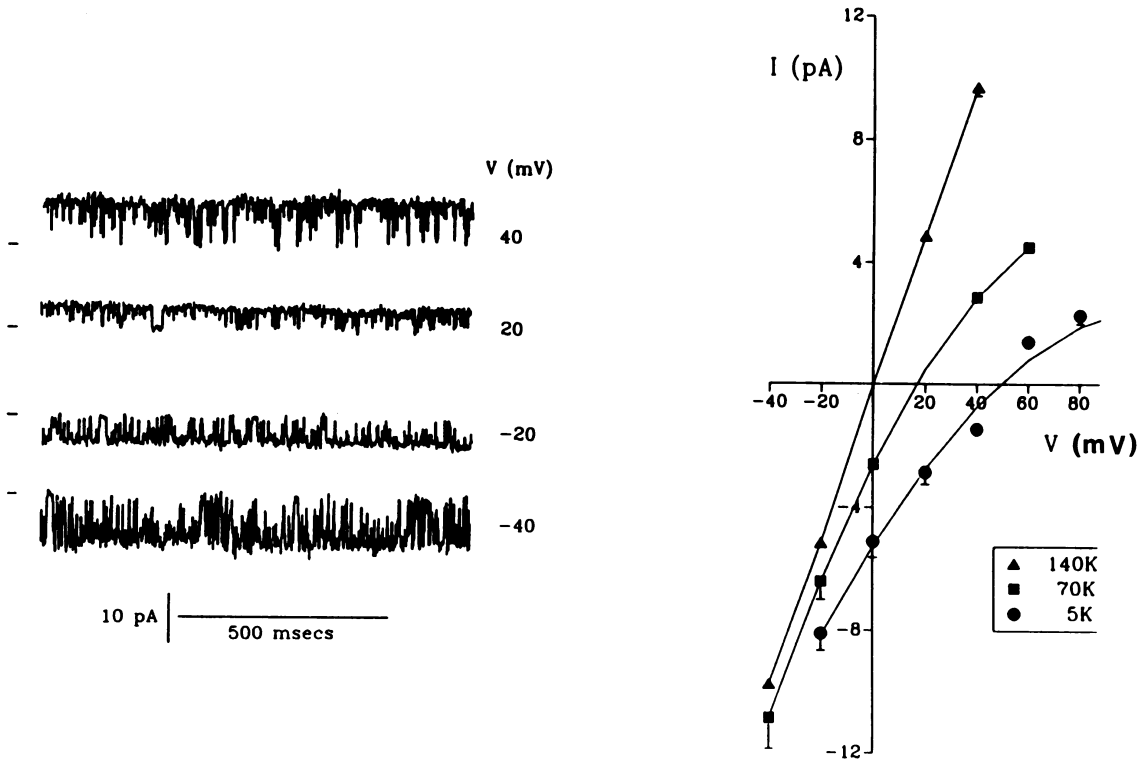

Fig. 3. Left: Excised patch recordings of channel currents at different applied potentials with symmetrical 140 mM K solution, containing 2 mM calcium on either side of the patch. Right: Current-voltage relations in excised patches where the K concentration in the bathing solution is reduced (in mM): from 140 K to 70 K to 5 K with 140 K in the patch pipette. In symmetrical solutions the unbroken line is a linear regression fit to the data. In the asymmetric solutions (5 and 70 mM K in the bath) the solid lines represent the best fit to the data using the Goldman-Hodgkin-Katz equation with a P_K:P_{Na} of 10 in both cases. The error bars are the SEM, some of which fall within the symbol itself. *V* is the applied voltage and corresponds to minus the potential applied to the pipette.

Table 2. Conductance and reversal potential for channel in excised patches with 140 mM K in the patch pipette and (in mM): 140 K, 70 K or 5 K in the bathing solution

Bath solution (mM)	*g* (pS)		E_{rev} (mV)	
	MG-63	SaOS-2	MG-63	SaOS-2
140 K	246 ± 4.5 (7)	223 ± 21 (3)	0.4 ± 0.4 (7)	3.7 ± 2.1 (3)
70 K	210 ± 7.3 (3)	191 ± 39 (3)	15.2 ± 2.0 (3)	17 ± 7 (3)
5 K	112 ± 7.4 (4)	132 ± 6 (5)	48.2 ± 1.6 (4)	49 ± 6 (5)

g is the channel conductance, E_{rev} is the reversal potential and the number between parenthesis is the number of experiments performed.

0.2). Repeating the same experiment with 1 μM calcium in the bath solution, where the channel was initially closed under control conditions (V_p = 0 mV), did not lead to an increase in P_o either (*data not shown*). In excised patches bathed in Ringer solution the channel is also insensitive to high concentrations of ATP. In control conditions P_o was 0.86 ± 0.02, and this was not significantly altered by the addition of 5 mM ATP to the bath solution (0.84 ± 0.02, n = 5 patches, $P > 0.09$).

To assess whether calcium influx is involved in the PTH or PGE_2-dependent K channel activation, $^{45}Ca^{++}$ uptake by MG-63 cells was measured in the presence and absence of either PTH or PGE_2 with or without 10 μM Nitrendipine. $^{45}Ca^{++}$ uptake increased from 2.74 ± 0.31 to 4.82 ± 0.45 nmol/mg protein/15 min (n = 6 experiments, $P < 0.01$) after preincubation with PTH and from 2.93 ± 0.32 to 5.20 ± 0.80 nmol/mg protein/15 min (n = 7 experiments, $P < 0.02$) after preincubation with PGE_2. In the presence of Nitrendipine alone, $^{45}Ca^{++}$ uptake was not significantly different from control. However, Nitrendipine inhibited the PTH- and PGE_2-stimulated $^{45}Ca^{++}$ uptake (PTH + Nitrendipine, 3.58 ± 0.5, n = 5

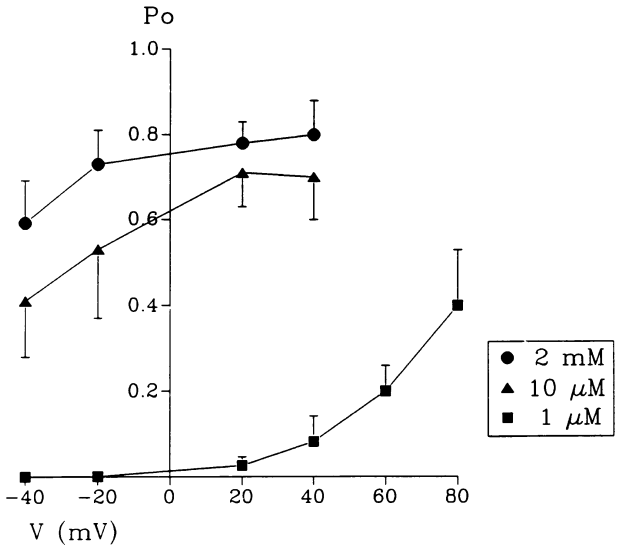

Fig. 4. Relationship between channel open probability (P_o) and voltage in symmetrical K solutions (140 mM) upon changes in bath calcium concentration (2 mM, 10 μM and 1 μM). The data are from 4 patches in which the effect of each different calcium concentration was assessed. The error bars are the SEM, some of which fall within the symbol itself. *V* is the applied voltage and corresponds to minus the potential applied to the pipette.

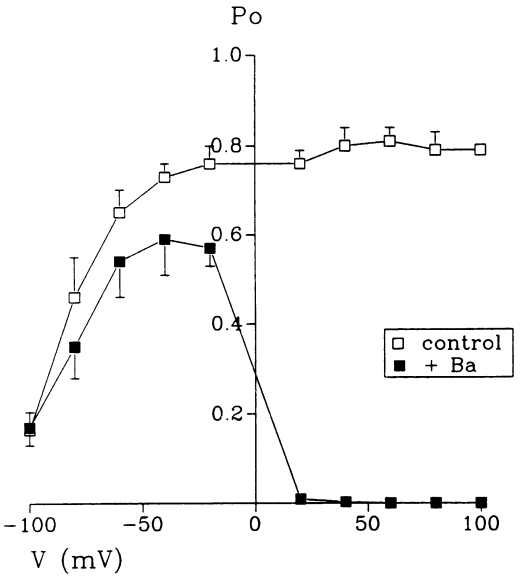

Fig. 5. Voltage-dependent barium block. One mM barium was added to the bath solution in excised patches with symmetrical K solution bathing the patch (140 mM K). Each point is the mean of 6 paired experiments. The error bars are the SEM, some of which fall within the symbol itself.

experiments, $P < 0.05$ *vs.* PTH alone; PGE_2 + Nitrendipine, 3.44 ± 0.42, $n = 6$ experiments, $P < 0.05$ *vs.* PGE_2 alone; both not significantly different from controls).

The effect of these PTH- and PGE_2-stimulated Ca influx on K channel activity were also tested using the patch clamp technique. In Ca Ringer, PGE_2 opened the K channel in 60% of the cases (7 out of 12 patches, Fig. 6), whereas in Ca-free Ringer this stimulation by PGE_2 was totally prevented (10 out of 10 patches). Similar results were also obtained for PTH and cAMP + forskolin (3 patches per condition, *not shown*). The presence of 10 μM Nitrendipine in the bath in combination with 2 mM Ca Ringer also prevented the opening of the channel by PGE_2 (5 out of 5 patches, Fig. 6) or cAMP + forskolin (2 patches, *not shown*). The presence of a K channel under the patch was verified in each case by excising the patch in a 2 mM Ca Ringer solution. Patches that did not show a maxi-K channel activity upon excision were discarded from this last analysis.

Discussion

This study shows that similar maxi-K channels are present on the plasma membrane of both MG-63 and SaOS-2 osteoblastlike cell lines. In MG-63 cells, this channel is activated in CAP upon stimulation of the cells with either PTH or PGE_2, but only when extracellular calcium was present. A similar activation was also observed by raising intracellular cyclic AMP with the external application of dibutyryl cAMP + forskolin. Possible mechanisms for the activation of this channel in silent CAP were investigated in the excised patch configuration. Interestingly, excision of either nonstimulated or stimulated patches yielded identical results, in that, a large conductance calcium-sensitive K channel was observed. This suggests that PTH, PGE_2 and dibutyryl cAMP + forskolin activate silent channels already present in the membrane rather than inducing the addition of new channels to the membrane.

In our experiments, since it is known that PTH and PGE_2 increase intracellular cyclic AMP in human derived osteoblast cells (MG-63) [27] as well as in other species [9, 30, 41, 48–50, 54], and since we observed a similar channel activation by artificially raising cell cyclic AMP with dibutyryl cAMP + forskolin, we investigated the possibility that the activation of the calcium-activated K channel was due to channel phosphorylation via activation of cyclic AMP-dependent protein kinase A. Such a mechanism has been shown to operate in pancreatic duct cells upon stimulation of the cells with a secretagogue [17]. In excised patches, either in low or high bath calcium, the catalytic subunit of PKA in the presence of ATP had no effect on channel P_o. Moreover, previous stimulation of the channel via PTH or PGE_2 in CAP had no subsequent effect on the P_o in excised patches whether these were treated or not with the catalytic subunit of PKA + ATP in the presence of either low or high calcium. This also suggests that previous phosphorylation via hormonal stimulation does not modify

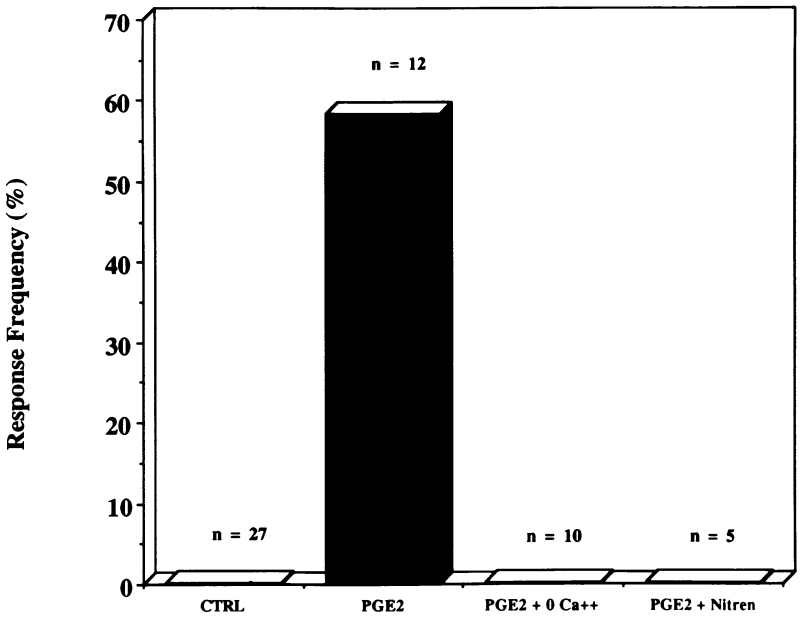

Fig. 6. The effect of calcium influx on K channel activation by PGE_2. The cell-attached patch recordings were taken after 2 min following addition of the different agents tested. At the end of the experiments, excision of the patch was performed to determine the presence of K channel under the patch. PGE_2 was used at 5 nM in the presence or the absence of 2 mM Ca in bath Ringer or in the presence of Ca and 10 μM Nitrendipine (Nitren). The total number of experiments is noted on top of the bars for individual assays.

the channel behavior nor its sensitivity to calcium. From this it was concluded that the mechanism of activation is not via direct channel phosphorylation by PKA. However, this does not exclude the possibility of phosphorylation of a soluble intermediary protein leading to channel activation, which is lost upon patch excision.

Another possible mediator of the PTH and PGE_2 response leading to channel activation is intracellular calcium. In excised patches, the channel is sensitive to calcium within the range of 1 to 10 μM, but the calcium sensitivity when the channel is exposed to normal cytoplasmic solutions is not known. Both PTH and PGE_2 are able to increase intracellular calcium concentration in a rat osteoblast cell line (UMR cells) [30, 40, 48–51]. If the same increase in intracellular calcium can be observed in our cells, then this could account for channel activation. Indeed, we show that $^{45}Ca^{++}$ uptake by MG-63 cells increased 1.8-fold in the presence of either PTH or PGE_2. In addition, this channel could not open in the absence of external calcium in the presence of PGE_2, PTH or cAMP + forskolin, nor if Nitrendipine was added in the bath solution containing 2 mM Ca. Hence, it is tempting to conclude from these results and from the available data in other osteoblast cells, that the activation of calcium-activated K channels in cell-attached patches by PTH and PGE_2 was due to an increase in intracellular calcium concentration, via an influx of calcium, as suggested in another cell system [47].

The activation of the calcium-activated K channel with dibutyryl cAMP and forskolin is now puzzling, since we would have to hypothesize that these agents directly increase intracellular calcium by activating a calcium channel [32, 51] or that cyclic AMP is able to mobilize calcium from intracellular stores [35, 42]. There is evidence for [40, 51] and against [30, 48] a rise in intracellular calcium upon increases in intracellular cyclic AMP in osteoblast cells elicited by either forskolin or dibutyryl cAMP.

In CAP, we obtained hormonal (PTH and PGE_2) activation of K channels in about 50 to 60% of assays where a channel was detected under the patch. This could either be due to the absence of receptors for PTH or PGE_2 in certain cells, the absence of Ca channels to permit Ca influx in these cells or the regulation of the above with the cell cycle. Recent data suggest that not all osteoblastlike cells possess receptors for PTH and PGE_2, and can increase intracellular calcium in response to these effectors [5, 31]. Indeed, intracellular free calcium responses to PTH and PGE_2 were noted in only 20 to 30% of UMR-106 cells, a rat osteosarcoma cell line [5]. In primary osteoblastlike fetal rat calvaria cell cultures, voltage-activated calcium channels were observed in 30% of the cells after 7 to 8 days in the presence of 10% FBS, whereas the presence of 2% FBS induced the expression of these channels within two days in 45% of the cells. Moreover, in UMR-106 cells the response to PTH is regulated differently throughout the cell cycle, as synchronization of these cells by a thymidine-aphidicolin block show variable intracellular free calcium response to this hormonal challenge in G1 and in S phase [2]. Hence, under our experimental conditions (2% FBS for 24 to 48 hr), the opening of large conductance K channels (via Ca influx) upon PTH or PGE_2 stimulation in 50 to 60% of the cells possessing K channels (and thus in about 25 to 30% of total cells) is very plausible. This would also indicate that maxi-K channels are activated only under certain conditions during the cell cycle as well.

Large conductance calcium activated K channels have been described in several types of cell preparations [1, 3, 4, 17, 20, 23, 24, 29, 34, 37–39, 43]. In the presence of physiological cytosolic calcium concentrations, these channels are almost always closed and are therefore thought not to contribute to the cell resting membrane potential. However, calcium-activated K channels are activated by membrane depolarization and elevation of intracellular calcium concentration into the micromolar or millimolar range [22, 29, 38]. Indeed, the K channel present in MG-63 cells was activated by micromolar calcium concentrations. In both MG-63 and SaOS-2 cells, this maxi-K channel has a slope conductance of 246 ± 4.5 and 223 ± 21 pS respectively, well within the measured conductances obtained for a number of different cell types [1, 29, 34, 38]. Most importantly, the characteristic slope conductance of the maxi-K channel in both MG-63 and SaOS-2 cells (*see* Table 2) is strikingly similar to those reported in the literature for chick primary osteoblastlike cell cultures [39] and pig primary chondrocyte cell cultures [15, 16]. Indeed, conductances of 114 and 112 pS were reported in asymmetrical bath (5 mM K) to pipette (140 mM K) conditions in excised patches for chick osteoblasts and pig chondrocytes respectively, whereas under symmetrical conditions (140 mM K in bath and pipette), conductances of 222 and 252 pS were noted in these cells [16, 39]. In addition, since the reversal potential was near zero in the excised configuration, in both MG-63 and SaOS-2 cells under symmetrical K conditions (140 mM) in the pipette and in the bath, and since the reversal potential was around −50 mV in the presence of 5 mM K in the bath, this suggests that the intracellular K concentration in these cells is elevated and near 140 mM, as suggested in other osteoblastlike cells [10, 39, 53].

The role of the maxi-K channel in MG-63 and SaOS-2 cells is still speculative. In MG-63 cells there are no data available as to the profile of membrane potential changes on addition of any of the agents that activated the calcium-activated K channel. Multiphasic membrane potential responses have been documented in different osteoblastlike cells in response to PTH [10, 12–14]. A common feature of this multiphasic response appears to be that PTH causes a long term hyperpolarization which can be mimicked by dibutyryl cAMP [12–14]. This hyperpolarization is thought to be caused by the opening of calcium-activated K channels in response to an increase in intracellular calcium concentration produced either by cAMP mediated activation of calcium channels [32, 51] or by cAMP mediated release of calcium from intracellular stores. If this hyperpolarization occurs in MG-63 cells, it could serve as a negative feedback controling a cyclic calcium movement across the membrane. Opening of voltage-dependent calcium channels by membrane depolarization, shown to be present in osteoblast cells [18, 19, 25] would increase intracellular calcium which in turn would activate the calcium activated K channels, hyperpolarizing the cell membrane and inactivating the calcium channels. Thus these K channels could act as a brake to calcium entry in these cells. Calcium-activated K channels in MG-63 and SaOS-2 cells could also be associated with one of the three types of mechanosensitive channels identified by Davidson et al. [6]. These channels show a slope conductance of 160 pS and are K selective in control conditions, whereas the conductances were 112 ± 7.4 and 132 ± 6 pS in MG-63 and SaOS-2 cells respectively under similar conditions. Furthermore, in thick ascending limb renal epithelial cells [45], membrane stretch is a physiological stimulation of calcium-activated K channels via a mechanism involving intracellular calcium variations. Hence, the presence of this calcium-activated maxi-K channel in MG-63 and SaOS-2 cells may also be involved in this type of signal transduction.

In conclusion, we have shown the presence of a calcium-activated K channel in human osteoblast cells (MG-63) which can be activated in cell-attached patches by PTH, PGE_2 and dibutyryl cAMP + forskolin. This channel is inhibited in a voltage-dependent manner by barium, sensitive to fluctuations in cytosolic calcium concentration, but insensitive to changes in cytosolic ATP or catalytic subunit of PKA. We propose that this channel is activated by an increase in intracellular calcium concentration upon stimulation by PTH and PGE_2 and suggest that it could be responsible for the long term hyperpolarization seen in other osteoblast cell types, thus acting to regulate, via changes in membrane potential, the intracellular calcium concentration.

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