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Parathyroid hormone activation of stretch-activated cation channels in osteosarcoma cells (UMR-106.01)

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Cell-attached patches of membrane of osteoblast-like cells UMR-106.01 respond to bath application of parathyroid hormone (PTH) with an increase in the average activity, as well as the single channel conductance, of a stretch-activated non-selective cation channel. Correlations with whole cell membrane potential and conductance changes are considered.

Osteoblastic cell line; Stretch-activated channel; Parathyroid hormone; Cyclic AMP

1. INTRODUCTION

Parathyroid hormone (PTH) and mechanical strain are critical factors in regulating bone modelling, in part through their actions on osteoblasts [1–3]. Osteoblasts, in turn, synthesize bone matrix proteins and prime bone matrix for targeting by osteoclasts. It is not known whether these chemical and physical factors work through entirely separate pathways or interact at some critical juncture. However, it is known that osteoblasts have mechano-sensitive or stretch-activated cation (C^+(SA)) channels [4,5], which, in other cells, appear to be tied to cytoskeletal elements [6]. Osteoblasts exposed to PTH respond in various ways including membrane depolarization [7,8] and cytosolic retraction [9,10]. Additionally, in other cells, C^+(SA) channels appear to be a target of action of growth-promoting hormones: platelet derived growth factor, for example, activates a C^+(SA) channel in fibroblasts [11]. Against this background, we investigated whether PTH might affect C^+(SA) channel gating in a clonal osteoblastic cell line (UMR-106.01), thereby making the C^+(SA) channel a site of convergence of two distinct osteoblast activator pathways.

2. MATERIALS AND METHODS

Subcultures of PTH-responsive UMR-106.01 cells (passages 9–16), originally derived from rat osteosarcoma, were grown to 40–80% confluence on glass coverslips, which were transferred to a recording chamber (1 ml volume) (Biophysica Technologies, Baltimore, MD) which permitted rapid bath solution change with minimal perturbation to the cells. Cells were bathed in either a mammalian Na^+ Ringer's (NR) consisting of (in mM): 140 NaCl; 5.5 KCl; 1 $MgCl_2$; 1 $CaCl_2$; 3 glucose, and 20 *N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid] (HEPES) buffer; titrated to pH 7.3 with NaOH or a K^+ Ringer's (KR) consisting of (in mM): 144 KCl; 1 $MgCl_2$; 1 $CaCl_2$; 20 HEPES; 3 glucose; titrated to pH 7.3 with KOH. Rat PTH (1–34 fragment), 8 br-cAMP or gadolinium (Sigma Chemical, St. Louis) were introduced to the chamber by perfusing the chamber with 10 ml of either NR or KR containing the final concentrations of these agents.

Standard electrophysiological techniques were used as previously adapted in our laboratory [4,12]. Single channel recordings were made with pipette solutions consisting of either KR or a Ca^{2+}-free K^+ Ringer's (KR0Ca) consisting of (in mM): 144 KCl; 1.3 EGTA; 20 HEPES; titrated to a pH of 7.3 with KOH. Single channel currents were filtered at 1 KHz and recorded at a sampling frequency of 3 KHz. The clamping potential V_c is defined as $-V_{pip}$, where V_{pip} is the potential imposed on the pipette interior with the bath taken as ground. Membrane potentials were most reliably recorded under current clamp conditions using 'perforated patch' techniques. For these experiments, the pipette solution consisted of (in mM): 12 NaCl; 64 KCl; 28 K_2SO_4; 47 sucrose; 1 $MgCl_2$; 0.5 EGTA; 20 HEPES, titrated to pH 7.35 with KOH. Nystatin was added at a concentration of 100 μg/ml to permeabilize the patch. Access resistances of <60 MΩ were sought. An interactive graphics program, which uses level crossings to determine in a segment of record the fraction of time when zero, one, or more channels are open, was used to measure the average number of channels open in a patch during a specified period of time (NP_o). Using a similar program, open channel amplitudes were determined and grouped into bins of 0.025 pA to produce amplitude histograms. Due to biological variability, comparisons were made between co-cultures of the same passage number.

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3. RESULTS

Figure 1A illustrates the effects of PTH on C^+(SA) channel activity in cell attached patches of UMR-106.01 membranes during application of suction to the interior of the pipette. PTH (50 nM), which has been shown to increase adenyl cyclase activity [13] and intracellular Ca^{2+} concentration [14] as well as depolarize the cell membrane [8], increased the mean activity (NP_o) of a channel which was open during application of suction

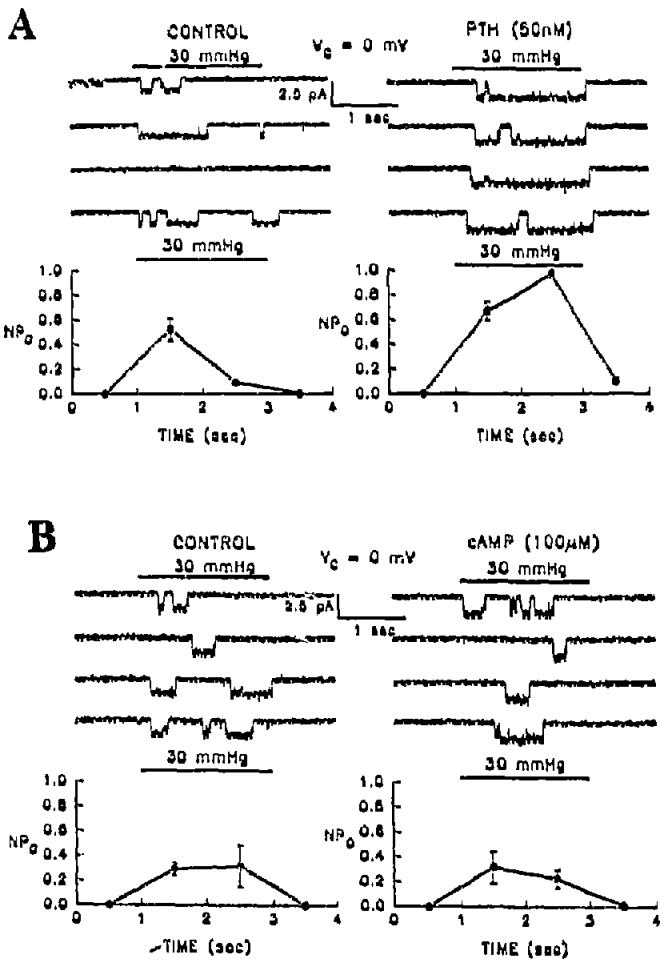

Fig. 1. Divergent effects of bath-applied PTH (A) and 8-br cAMP (B) on C^+(SA) channels in UMR-106.01 cells bathed in NR. Cell attached patches held at pipette potential of 0 mV for 6 s intervals with suction (30 mmHg) applied to the pipette during the third and fourth seconds. (A) Sample single channel current traces and mean channel activity determined on the same patch using identical voltage and suction sequences before (control) and 2 min after addition of PTH to the bath. Under control conditions, NP_o was constant through the duration of stretch (0.225 ± 0.048). PTH significantly increased NP_o to 0.446 ± 0.066 (P<0.001) during the 2 min immediately following PTH addition in 68% of the patches (10 of 15). (B) Sample single channel traces and mean channel activity determined as in (A) for control and 8 br-cAMP treated C^+(SA) channels. During the control period NP_o was 0.212 ± 0.062. Following addition of 8 br-cAMP, NP_o was not significantly altered, averaging 0.193 ± 0.058 (n=5).

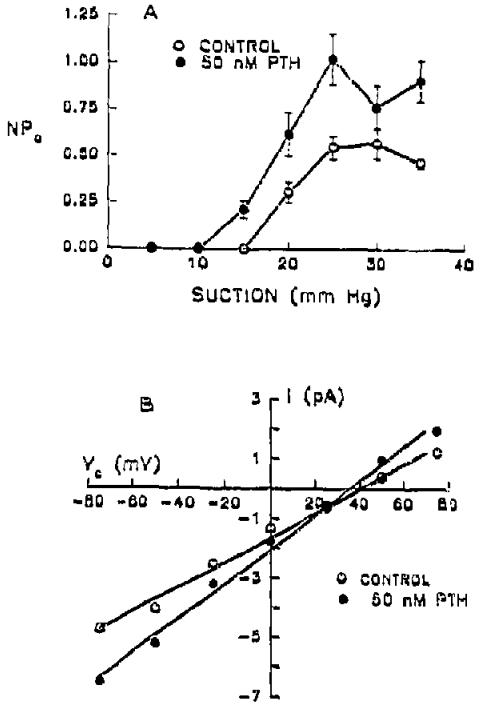

Fig. 2. Effects of PTH (50 nM) on the stretch sensitivity and single channel conductance of C^+(SA) channel. (A) Plot of average activity vs. applied pipette suction demonstrating that PTH reduced the threshold level of suction required to activate the C^+(SA) channels without altering the amount of suction required to obtain maximal levels of activity. (Experiment typical of a series of four.) (B) Single channel current vs. voltage curves demonstrating increase in single channel conductance after bath application of PTH. Small shift in zero current potential was not statistically significant.

to the pipette. In the presence of PTH, note that on repeated application of suction, the single stretch-activated channel present in the patch opens every time suction is applied, spends much of the duration of the suction pulse in the open state and then closes down promptly with cessation of suction. This channel has previously been shown to be a non-selective cation channel in that it selects cations over anions but does not select between Na^+ and K^+ and passes Ba^{2+} [4]. It also shows little consistent voltage dependence over a 100 mV range around the resting membrane potential of the cell. In Fig. 1B, the same experimental protocol was repeated, but following application of 100 μM 8 br-cAMP, a membrane permeant cAMP analogue. Note that after addition of up to 1 mM 8 br-cAMP there is no obvious or computed change in mean channel activity evoked by stretch.

Figure 2A and 2B compares the stretch sensitivity and current/voltage (i–V) characteristic of the C^+(SA) channel prior to and after addition of PTH. These experiments and the remaining single channel studies were conducted with very low $Ca^{2+}{}_o$ in the pipette. Low $Ca^{2+}{}_o$ increases the peak conductance of the C^+(SA) channel from 19.7 ± 1.9 pS (n=12) to 41.2 ± 3.8 pS (n=6) (P<0.001) thereby amplifying changes in channel amplitudes and conductance. Figure 2A demonstrates that addition of PTH reduces the threshold level of suction required to activate the C^+(SA) channel. However, even though PTH enhanced the mean activity of the channel by 70%, the amount of suction required to obtain maximal levels of activity was not different from control. Figure 2B demonstrates that PTH increased the single channel current amplitude seen at patch potentials near or hyperpolarized to the resting potential of the patch

(see also Fig. 1A). With Ca^{2+}_o present in the pipette, channel amplitudes were also increased with PTH, suggesting that Ca^{2+} was not a factor in the PTH-induced change in conductance. As multiple forms of mechanosensitive channels have previously been seen in osteoblast-like cells [5], we tested the possibility that the increase in single C^+(SA) channel conductance resulted from the condition that the C^+(SA) channel could occupy several conductance states and that the larger states were preferred in the presence of PTH. To do this we examined single channel current amplitudes (i) in the presence of both very low Ca^{2+}_o in the pipette and high K^+ Ringer's in the bath. High K^+ Ringer's in the bath prevents small shifts in i due to cell depolarization (see sample trace Fig. 3A). Figure 3A displays channel currents before and after PTH, while 3B shows histograms of open channel current amplitudes. In this cell, typical of 8 out of 12 cells, single channel current amplitudes were well fit to Gaussian distributions, suggesting one predominant conductance level for the channel in the presence or absence of PTH. Note however, that the peak amplitude for the open state is shifted from 1.25 pA in control conditions to 1.63 pA during PTH exposure. This increase in channel conductance was consistent in all experiments (n=12). Small fluctuations in current were occasionally visible at the leading or trailing edge of a burst of channel activity suggesting that the channel may open or close through a transient subconductance state(s). However, given the long channel open times, brief sojourns at these subconductance levels, highlighted by arrows in Fig. 3A, would be expected to contribute little area to the amplitude histograms.

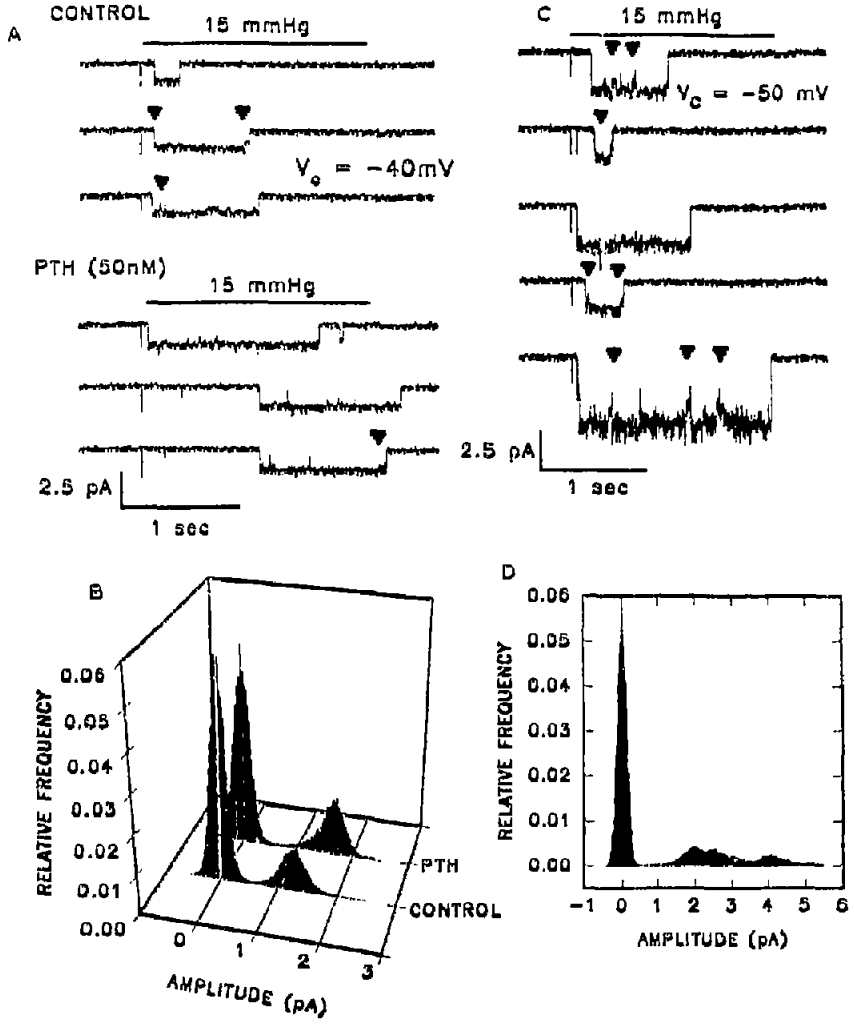

Fig. 3. Effects of PTH on single channel amplitudes of the C^+(SA) channel. (A) Traces of single channel currents recorded before and after addition of PTH to the high K^+, low Ca^{2+} bath to null out the cell resting potential. Arrows indicate that the channel may open or close through a transient subconductance state which contributes little to the amplitude histogram. (B) Amplitude histograms for single C^+(SA) channel currents recorded before and after addition of PTH. Currents were recorded during suction pulses of 15 mmHg with the patch held V_c = −40 mV. Note the increase in peak channel amplitude from 1.25 pA to 1.63 pA after treatment with PTH. (C) Single channel tracings from a representative experiment demonstrating intermediate conductance states (arrows). (D) Amplitude histogram for single channel currents. Note the non-Gaussian distribution of channel openings.

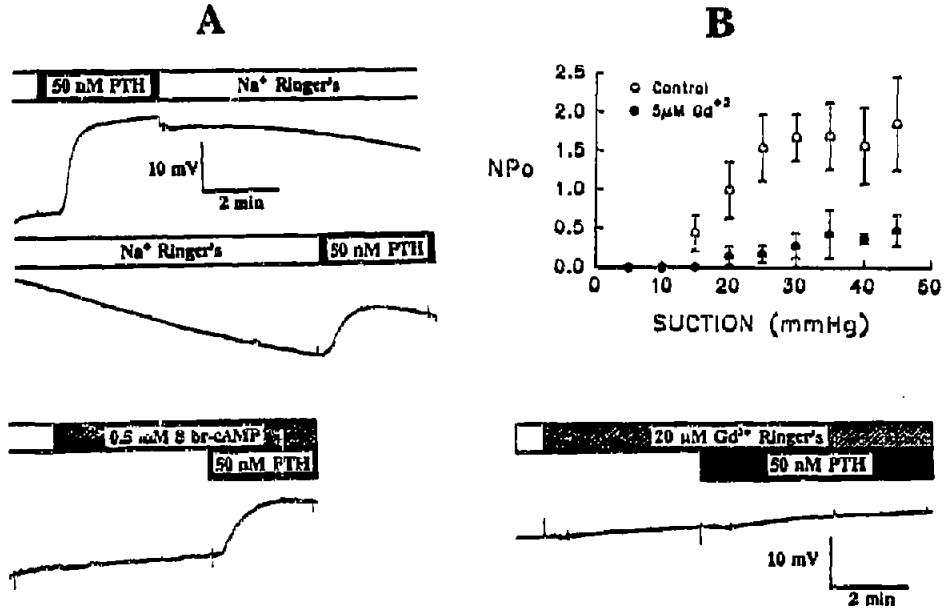

Fig. 4. Correlation of single C^+(SA) channel activity with whole cell membrane potential. (Panel A, *upper traces*): PTH-induced depolarization occurring within 1–2 min after addition, followed by slow repolarization with washout in Ringer's. Reapplication of PTH resulted in a second depolarization. This was atypical, being seen in only 2 out of 8 PTH responsive cells. (A, *lower trace*): lack of effect of 0.5 mM 8 br-cAMP on the membrane potential (typical of an n=6), while subsequent application of PTH produced a substantial depolarization. (B, *upper panel*): effects of gadolinium on single C^+(SA) channels in the cell attached patch. Channel activity was compared between patches in the same cell. Addition of 5 μM Gd^{3+} to the pipette solution significantly decreased NP_o from average maximum levels of 1.707 ± 0.071 to 0.429 ± 0.039 (n=7; P<0.001) without affecting the stretch sensitivity. (B, *lower trace*): block of PTH-induced membrane depolarization by pre-treatment with 20 μM gadolinium. Trace is typical of 6 similar experiments.

However, in 4 experiments, (e.g. Fig. 3C) frequent sojourns into a subconductance state were noticeable and open channel histograms were not well fit to a Gaussian distribution. Even in these experiments, PTH shifted the majority of channel openings to higher amplitudes. Interestingly, addition to the bath of 100 μM 8 br-cAMP, which failed to increase channel activity, none-the less mimicked the PTH-induced increase in channel conductance (data not shown).

If the effects of PTH on C^+(SA) channel activity in the cell-attached patch of membrane were representative of that of C^+(SA) channels in the remainder of the cell and C^+(SA) channels contributed substantially to the whole cell membrane conductance, then we might predict that addition of PTH would depolarize the UMR-106.01 cell membrane and increase its conductance. Using this line of reasoning, an agent which blocked the C^+(SA) channel might be expected to substantially reduce the effect of PTH on membrane potential and conductance. Fig. 4 provides tests of both of these predictions.

In Fig. 4A, it is apparent that addition of PTH (50 nM) to the Ringer's bath resulted in rapid membrane depolarization. In the perforated patch configuration, 8 out of 12 cells responded within 1–2 min to 50 nM PTH with an average 11.3 ± 2.5 mV depolarization (range 6.0–23.2 mV) which slowly reversed after washout. Similar results were also seen in conventional whole cell current clamp recording. The membrane potential was not measureably altered by bath application of 0.5 mM 8 br-cAMP (lower trace, panel A). Fig. 4B demonstrates that trivalent cation gadolinium (Gd^{3+}), which when added to the patch pipette at 5 μM more than halves C^+(SA) channel activity in the cell-attached patch (top panel), prevents PTH-induced depolarization at 20 μM. Gadolinium has been shown to block stretch-activated channels with similar characteristics to the C^+(SA) channels in *Xenopus* oocytes [15].

4. DISCUSSION

We have obtained evidence that bath application of parathyroid hormone, in concentrations which effect other cell functions, results in an increase in activity of a stretch activated non-selective cation (C^+(SA)) channel seen in cell-attached patches of membrane from a PTH-responsive osteoblast-like cell line UMR-106.01. This effect is accompanied by a small increase in single channel conductance. These observations suggest that the C^+(SA) channel might be a locus at which the actions of membrane deformation and PTH converge. This interpretation is supported by evidence that PTH often depolarizes the cell membrane while pretreatment with Gd^{3+}, which reduces the activity of C^+(SA) channels in the cell-attached patch, prevents PTH-induced depolarization. However, our attempts to measure changes in membrane conductance underlying the de-

polarization, have produced scattered results which are not highly consistent with the C^+(SA) channel being the sole or major electrophysiological target of PTH action. It is possible that PTH also effects other ion channels in the membrane. For example, by simultaneously closing a channel with a reversal potential negative to the cell's measured resting potential of −30 to −40 mV, while opening the C^+(SA) channel, PTH could induce depolarization with very variable effects on membrane conductance. Recently, the physiological activity of stretch-activated channels has been questioned in other cells due to the inability to see macroscopic conductances activated by cell deformation which parallel the activity of stretch-activated channels recorded in the cell-attached patch [16]. More rigorous correlation of macroscopic and single channel currents shall be critical in clarifying these points. Analysis of results of microelectrode studies in the UMR-106.06 cell line, using specific channel antagonists, are consistent with PTH producing the membrane depolarization by promoting the closure of Ca^{2+}-activated K^+ channels [7,8]. Other studies with whole cell recordings suggest that PTH activates chloride currents in osteoblasts [17]. Additionally, PTH has been shown to inhibit L-type Ca^{2+} channels in the neurobiastoma cell [18]. However, in pilot experiments done in conjunction with the current studies, neither nitrendipine, an L-type Ca^{2+} channel blocker, nor Ba^{2+}, a K^+ channel blocker, prevented PTH-induced depolarization.

The mechanism of action of PTH on C^+(SA) channels in UMR-106.01 cells, as well as the identity of possible second messengers involved, is currently unknown. PTH regulation of osteoblast function is generally thought to be mediated through the adenylate cyclase pathway. However, in our experiments, the effect of PTH on mean channel activity is not duplicated by bath application of a membrane permeant analog of cAMP, although the smaller effect of PTH on single channel conductance is modulated by 8 br-cAMP. These data suggest that PTH stimulation of the C^+(SA) channel occurs through a different second messenger pathway. PTH has been shown to elevate inositol polyphosphates and diacylglycerides and stimulate the phospholipase C pathway [14,19] in cells displaying osteoblast phenotypes. Interestingly, when cultured osteoblasts are subjected to mechanical stress, intracellular concentrations of inositol phosphates, as well as cAMP, are elevated [20].

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