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The effects of parathyroid hormone on L-type voltage-dependent calcium channel currents in vascular smooth muscle cells and ventricular myocytes are mediated by a cyclic AMP dependent mechanism

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The present study demonstrated that L channel currents were decreased in smooth muscle cells, and increased in ventricular myocytes by both bovine parathyroid hormone, (bPTH-(1–34)), and dibutyryl cyclic AMP (db-cAMP), using the whole cell version of the patch clamp technique with Ba^{2+} as the charge carrier. The effects of bPTH-(1–34) and db-cAMP on L channel currents were additive but not synergistic. Furthermore, the effects of bPTH-(1–34) on L channel currents in these 2 cell preparations were abolished in the presence of a cAMP antagonist. These results suggest that the effects of bPTH-(1–34) on L channel currents in vascular smooth muscle cells and ventricular myocytes are mediated by a cAMP-dependent mechanism.

Calcium channel; Parathyroid hormone; Cyclic AMP; Vascular smooth muscle cell (rat); Ventricular myocyte (rat)

1. INTRODUCTION

Parathyroid hormone (PTH) has vasodilatory and cardiotonic effects. Recently, it has been shown that these effects of PTH can be related to the modulation of L-type calcium channel currents by this hormone. The bovine PTH fragment composed of N-terminal amino acids from 1 to 34, bPTH-(1–34), inhibits L channel currents in vascular smooth muscle cells [1] while it increases L channel currents in ventricular myocytes [2]. The underlying mechanism concerning these tissue selective effects of PTH on L channel currents is not clear.

In both vascular smooth muscle [3,4] and cardiac muscle [5], PTH increases cyclic AMP (cAMP) content. It is well documented that the increase in cAMP content plays an important role in the vascular effect of PTH [6]. In isolated vascular smooth muscle cells, the increase in cAMP content occurred within 1 min and was maximal within 5–10 min after treatment with bPTH-(1–34) [7]. The relaxation of vessel strips induced by bPTH-(1–34) in the above study was immediate but the maximum effect of PTH did not develop until 6 min [7]. In agreement with this, a period of 5 min was also needed to establish the inhibitory effect of bPTH-(1–34) on L channel currents from rat tail artery smooth muscle cells in our previous investigation (unpublished observation). In cardiac tissue, PTH also enhanced cAMP content prior to its chronotropic action [5]. The similarity in the temporal sequence of PTH effects on cAMP content, calcium channel currents and cardiovascular responses suggests the hypothesis that the PTH effect on L channel currents may be mediated, at least in part, by a cAMP-dependent mechanism. Direct electrophysiological evidence derived from the present study indicates that cAMP is involved in the modulation of L channel currents by PTH in both vascular smooth muscle cells and ventricular myocytes.

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2. MATERIALS AND METHODS

Single vascular smooth muscle cells were isolated from the tail artery of male Sprague–Dawley rats (100–200 g body wt) [8]. The cells were cultured in a CO_2 incubator at 37°C and were used within 8–36 h after they were plated. Ventricular myocytes from neonatal rat (3 days old) were dispersed according to a modification of the method of Yatani and Brown [9]. The harvested myocytes grew into a confluent beating monolayer after a 24 h incubation (37°C, 5% CO_2, 95% humidified air). The voltage clamp studies were carried out before the cell monolayer was formed.

The whole-cell version of the patch clamp technique was used to measure the inward currents [10]. The bath solution contained (mM): Tris, 110; CsCl,5; HEPES,20; glucose, 30; $BaCl_2$,20; KCl,5; and TTX,0.5 μM. The pipette solution contained (mM): Cs_2-aspartate,75; EGTA,10; ATP,2; $MgCl_2$,5; K-pyruvate,5; K-succinate,5; glucose,25; HEPES,15; creatinphosphate-Na_2,5; and creatin-kinase, 50 units/ml (Boehringer Mannheim Gmbh Co., FRG). The pH and osmolality of both bath and pipette solutions were adjusted to 7.4 and 320 mOsm, respectively. Linear leakage and capacitive currents were subtracted on-line with the use of software (pClamp). Series resistance was compensated electronically using the Axopatch-1B (Axon Instruments, Inc., USA). Currents were filtered with a 4-pole Bessel filter at a cutoff frequency of 5 kHz. The experiments were carried out at room temperature (20–22°C).

bPTH-(1-34) was purchased from Bachem Inc. (USA). It was dissolved in distilled water and 15 μl fractions of the stock solution of 2.43×10^{-4} M were stored at -80°C. Dibutyryl adenosine 3':5'-cyclic monophosphate (db-cAMP) and imidazole were purchased from Sigma (USA). Rp-adenosine 3':5'-cyclic monophosphothioate, Rp-cAMPs, was from Biolog Life Sci. Ins. (FRG). The final concentration of bPTH-(1-34) in the bath was achieved by a single addition in order to avoid possible desensitization of the cell to the peptide.

The paired Student's *t*-test or group *t*-test was used for comparison between mean values of the control and those obtained after drug administration. In the case of multiple comparisons, analysis of variance in conjunction with the Newman-Keul's multiple range test was applied. Values of $P<0.05$ were considered statistically significant.

3. RESULTS

3.1. *Effects of db-cAMP on L channel currents*

db-cAMP was added to the bath solution to achieve a final concentration of 1 mM. At this concentration, db-cAMP decreased L channel currents in vascular smooth muscle cells. The kinetics and I-V relationship of L channel currents were not changed in the presence of db-cAMP (Fig. 1A). In 9 cells, db-cAMP at a concentration of 1 mM induced a 26% decrease in L channel current amplitude ($P<0.05$). Furthermore, the smooth muscle cells were incubated for more than 20 min in the presence of 1 mM imidazole, a phosphodiesterase stimulator [3], and then exposed to bPTH-(1-34). The amplitude of L channel currents were -79 ± 8 pA after pretreatment with imidazole and -73 ± 6 pA after the sequential application of 1 μM bPTH-(1-34) ($n=9$, $P>0.05$).

In ventricular myocytes, 1 mM db-cAMP increased L channel currents. The peak amplitudes of L channel currents were -365 ± 87 and -428 ± 92 pA before and after the application of db-cAMP ($n=7$, $P<0.05$). Furthermore, the peak of the I-V relationship of L channel currents was shifted toward the direction of hyperpolarization by 10 mV, which was confirmed in another 4 cells. bPTH-(1-34) also induced a negative shift of the I-V relationship of L channel currents (data not shown).

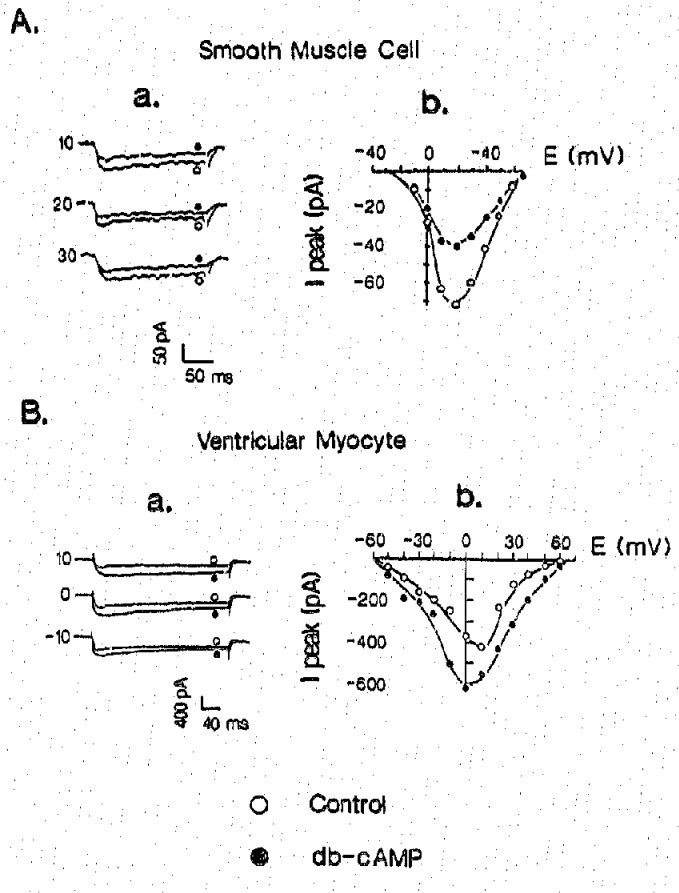

Fig. 1. The effect of db-cAMP on L channel currents in one vascular smooth muscle cell (A) and one ventricular myocyte (B). L channel currents were elicited from a holding potential of -40 mV to various test pulses. Original current traces, shown in A-a and B-a, in the absence (open circles) and then presence (filled circles) of db-cAMP (1 mM) were obtained at various test pulses indicated beside each record (mV). The I-V relationships of L channels, shown in A-b and B-b, were obtained before and after the application of db-cAMP.

3.2. *The additive effects of db-cAMP and bPTH-(1-34) on L channel currents*

As shown in Fig. 2, the sequential application of db-cAMP and bPTH-(1-34) produced an additive effect on

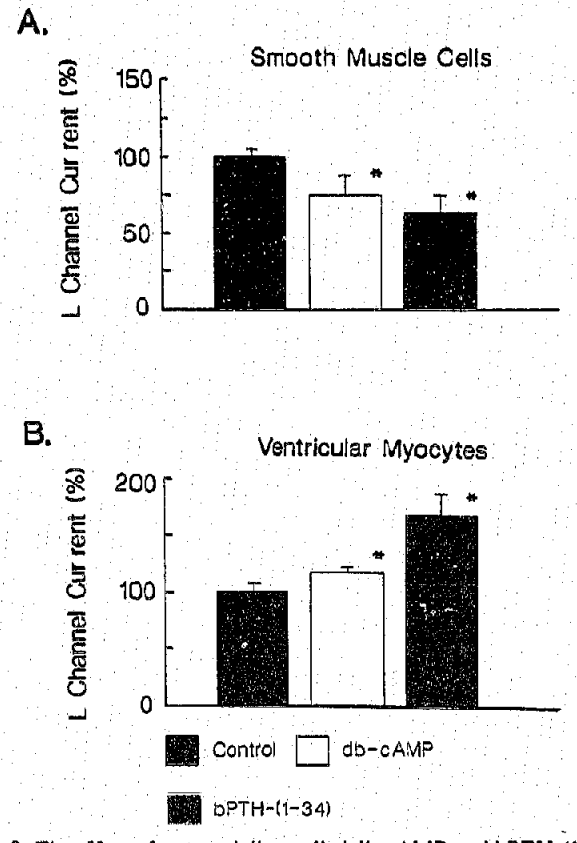

Fig. 2. The effect of sequentially applied db-cAMP and bPTH-(1-34) on L channel currents in vascular smooth cells (A) and ventricular myocytes (B).

L channel currents. In vascular smooth muscle cells (Fig. 2A), after 24.6% inhibition by 1 mM db-cAMP, 1 μM bPTH-(1-34) induced an additional 12% inhibition in L channel currents. The total inhibition of L channel currents induced by db-cAMP and bPTH-(1-34) was 36.8% ($n = 5$) which was approximately the same as the value in the presence of 1 μM bPTH-(1-34) alone [1,2]. Since the effect of bPTH-(1-34) on L channel currents was almost saturated at a concentration of 1 μM (data not shown), this result suggested that the effects of db-cAMP and bPTH-(1-34) were additive but not synergistic. The increase in L channel currents in the presence of 1 mM db-cAMP was further enhanced by the application of 1 μM bPTH-(1-34).

In ventricular myocytes (Fig. 2B), 1 mM db-cAMP increased L channel currents by 17 ± 4%. Subsequent application of bPTH-(1-34) at the maximal effective concentration of 1 μM only increased L channel currents by an additional 51% ($n = 5$, $P < 0.05$) (Fig. 2B). As reported previously, 1 μM bPTH-(1-34) administered alone maximally increased L channel currents by 68% ($n = 13$, $P < 0.01$) [2].

3.3. *The antagonization of bPTH-(1-34) effects by Rp-cAMPs*

When 100 μM Rp-cAMPs, which is a cAMP antagonist, was included in the pipette solution, the inhibitory and excitatory effects of bPTH-(1-34) on L channel currents in vascular smooth muscle cells (Fig. 3) and ventricular myocytes (Fig. 4), respectively, were abolished. It is interesting that after the intracellular infusion of Rp-cAMPs, the amplitude of L channel currents increased by a small amount in response to bPTH-(1-34) ($n = 5$, $P < 0.05$) in vascular smooth muscle cells (Fig. 3B). In ventricular myocytes, the amplitude of L channel currents decreased by 14% in the presence of 1 μM bPTH-(1-34) with Rp-cAMPs infusion ($n = 7$, $P < 0.05$) (Fig. 4B). In addition, the kinetics of inactivation of L channel currents were changed by bPTH-(1-34) with Rp-cAMPs infusion (Fig. 4A-a). This could

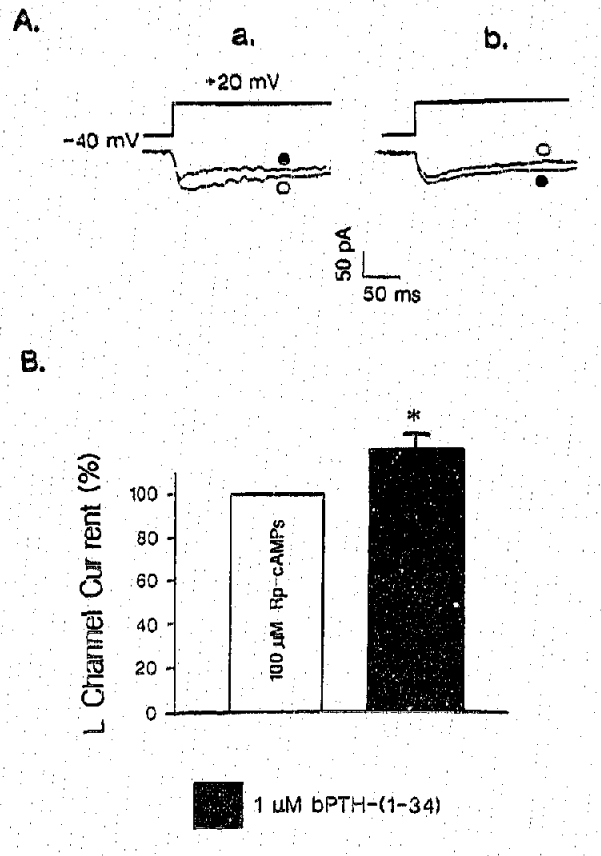

Fig. 3. Modification of bPTH-(1-34) effects by the intracellular infusion of Rp-cAMPs in vascular smooth cells. A. The original current traces in the absence (open circles) and then presence (filled circles) of 1 μM bPTH-(1-34). The records shown in A-a were taken from a cell without Rp-cAMPs in the pipette solution. The records shown in A-b were taken from another cell with Rp-cAMPs in the pipette solution. B. With 100 μM Rp-cAMPs in the pipette solution, the effect of bPTH-(1-34) on L channel currents was abolished ($n = 5$, $P < 0.05$).

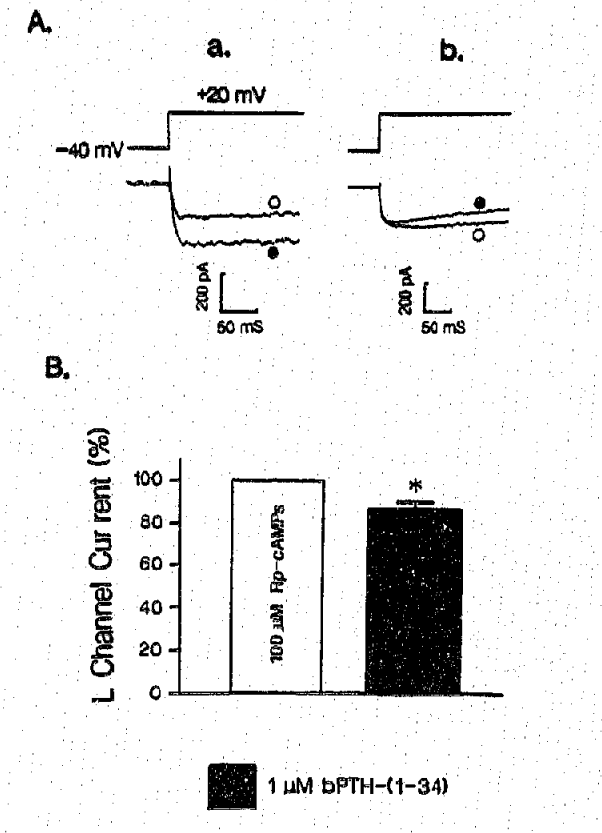

Fig. 4. Modification of bPTH-(1-34) effects by the intracellular infusion of Rp-cAMPs in ventricular myocytes. (A) The original current traces in the absence (open circles) and then presence (filled circles) of 1 μM bPTH-(1-34). The records shown in A-a were taken from a cell without Rp-cAMPs in the pipette solution. The records shown in A-b were taken from another cell with Rp-cAMPs in the pipette solution. (B) With 100 μM Rp-cAMPs in the pipette solution, the effect of bPTH-(1-34) on L channel currents was abolished ($n = 7$, $P < 0.05$).

not be observed without Rp-cAMPs in the pipette (Fig. 4A-b).

4. DISCUSSION

When db-cAMP was applied to the bath solution, this non-hydrolysable but permeable cAMP analog mimicked every aspect of the effects of bPTH-(1-34) on L channel currents in both vascular and ventricular muscle cells. A non-synergistic effect of db-cAMP and bPTH-(1-34) further suggested that these 2 agents used the same subsequent pathway. As an analogy, the effects of isoproterenol and cAMP were also not synergistic [11]. Finally, an antagonist of cAMP, Rp-cAMPs, completely abolished the effect of bPTH-(1-34) on L channels [12]. Taking all this evidence together, it is apparent that bPTH-(1-34) effects on L channel currents are mediated by a cAMP-dependent mechanism.

It has been suggested that an increase in cAMP content in vascular smooth muscle cells may decrease intracellular Ca^{2+} by activation of cyclic GMP (cGMP)-dependent protein kinase. On the other hand, activation of cAMP-dependent protein kinase by cAMP would result in increased entry of extracellular Ca^{2+} [13]. The presence of both cAMP- and cGMP-dependent protein kinases in primary cultured vascular smooth muscle cells has been demonstrated [13]. Hence, the effect of bPTH-(1-34) on L channel currents in vascular smooth muscle cells could be the result of the predominant activation of cGMP-dependent protein kinase, which was secondary to the increase in the cAMP content stimulated by bPTH-(1-34). If Rp-cAMPs could bind competitively to cGMP-dependent protein kinase, the Rp-cAMPs induced inhibition of the effect of bPTH-(1-34) on L channel currents would be explained. Such binding has not been reported.

In cardiac and many other tissues, cGMP-dependent protein kinase was absent or the concentration was too low to be routinely detectable [14-16]. Furthermore, cGMP had no effect on calcium channel currents in single ventricular cells [17]. Although a more potent analogue of cGMP, 8-bromo-cGMP, has been reported to inhibit the calcium channel currents in embryonic chick ventricular myocytes [18], this inhibitory effect could be interpreted as an increased cAMP hydrolysis via a cGMP-stimulated cyclic nucleotide phosphodiesterase [19-21]. In the present study, when cAMP concentration was increased by bPTH-(1-34), it probably only activated a cAMP-dependent protein kinase in cardiac tissues, and, hence, an increase in L channel currents in ventricular myocytes occurred. This hypothesis needs more experimental evidence and at this point is only speculation.

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