Journal of Physiology (1991), **441**, *pp.* 325–346
With 9 figures
Printed in Great Britain

PARATHYROID HORMONE SELECTIVELY INHIBITS L-TYPE CALCIUM CHANNELS IN SINGLE VASCULAR SMOOTH MUSCLE CELLS OF THE RAT

BY RUI WANG, EDWARD KARPINSKI AND PETER K. T. PANG

From the Department of Physiology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

(*Received 17 September 1990*)

SUMMARY

1. The active synthetic N-terminal fragment of bovine parathyroid hormone, bPTH-(1–34) at a concentration of 1 μM, decreased the peak amplitude of the long-lasting (L-type) calcium channel current by 37% ($n = 14$, $P < 0{\cdot}01$) in rat tail artery smooth muscle cells. By contrast, this fragment of parathyroid hormone (PTH) (1 μM) had no effect on the transient (T-type) calcium channel current in the same cell preparation.

2. The inhibitory effect of bPTH-(1–34) on L-channel currents was reversible and could be antagonized by the L-channel agonist, Bay K 8644. In contrast, bPTH-(1–34) inhibited Bay K 8644-induced amplification of L-channel currents.

3. The inhibitory effect of bPTH-(1–34) on L-Channel currents was dose dependent with a threshold concentration of less than 10^{-7} M, and voltage dependent with increased inhibition at more positive holding potentials. However, this effect of bPTH-(1–34) was not dependent on different pulse lengths or interpulse intervals.

4. The kinetics of deactivation of L-channel currents were not changed although the instantaneous amplitude of the L-channel tail current was reduced by bPTH-(1–34).

5. Application of bPTH-(1–34) antagonists (10^{-6} M-bPTH-(3–34) and 10^{-5} M-bPTH-(7–34)) did not result in any significant change in the magnitude of L-channel currents ($n = 15$ and $n = 7$, respectively).

6. Pre-incubation of cells with bPTH-(3–34) for more than 15 min abolished the inhibitory effect of bPTH-(1–34) on L-channel currents.

7. The present study provides direct evidence to demonstrate that PTH, an endogenous circulating hormone, is a selective inhibitor of L-channel currents in vascular smooth muscle cells.

INTRODUCTION

Control of many Ca^{2+}-dependent cellular events in excitable cells may depend on the modification of calcium influx via voltage-dependent calcium channels. Investigations using the patch-clamp technique have identified two types of voltage-dependent calcium channel currents in rat tail artery smooth muscle cells (Wang, Karpinski & Pang, 1989). The transient (T-type) calcium channel current is

activated at negative potentials and inactivates quickly and completely, while the long-lasting (L type) calcium channel current is activated at more positive membrane potentials and shows minimal inactivation. It has been suggested (Benham, Hess & Tsien, 1987) that the L-channels represent the main population of voltage-dependent calcium channels in vascular smooth muscle cells and these channels may play a major role in the movement of Ca^{2+} into the cell to trigger muscle contraction (Ohya & Sperelakis, 1989).

The mechanisms by which the voltage-dependent calcium channels are modulated are not clear. Synthetic calcium antagonists have been used experimentally and clinically for more than a decade (Greenberg, 1987; Bean, 1989). Only recently, however, has the modulatory effect of endogenous hormones and peptides on calcium channels received attention. This may be a physiologically important mechanism. Parathyroid hormone (PTH), for example, has been implicated in the modulation of voltage-dependent calcium channels in many cell types. As a potent vasodilator, bovine PTH (1–34) (bPTH-(1–34)) inhibited KCl-stimulated vasoconstriction and $^{45}Ca^{2+}$ uptake in rat tail artery strips (Pang, Hong, Yen & Yang, 1984; Pang, Yang & Sham, 1988). In guinea-pig papillary muscles, bPTH-(1–34) increased the peak amplitude of a slow inward current by 37% (Kondo, Shibata, Tenner & Pang, 1988). However, in neuroblastoma cells, bPTH-(1–34) was reported to be a specific inhibitor of L-channel currents (Pang, Wang, Shan, Karpinski & Benishin, 1990). In that study, Pang *et al.* (1990) also reported a preliminary result which was that bPTH-(1–34) decreased the peak amplitude of L-channel currents in rat tail artery smooth muscle cells. This effect of PTH on voltage-gated Ca^{2+} channels may provide an explanation for the PTH-induced vasorelaxation. The focus of the study by Pang *et al.* (1990) was the effect of bPTH-(1–34) on calcium channel currents in neuroblastoma cells. The indication derived from this preliminary study on a small number of smooth muscle cells was that bPTH-(1–34) inhibited Ca^{2+} channel currents. However, the mechanisms of the action of PTH on voltage-dependent Ca^{2+} channels in vascular smooth muscle cells still remain to be elucidated. In the present study, the specificity, reversibility, membrane potential dependence and concentration dependence of the PTH effect are examined. The effect of bPTH-(1–34) on the inactivation and deactivation of L-channel currents was also studied. In addition, the effects of different fragments of PTH (bPTH-(3–34) and bPTH-(7–34)) and their interaction with bPTH-(1–34) were investigated. This study, then, considerably extends the previous preliminary report and describes the mechanisms of action of PTH on Ca^{2+} channels in rat tail artery smooth muscle cells.

METHODS

Cell preparation

Single smooth muscle cells were dispersed enzymatically following the procedures of Wang *et al.* (1989) with some modifications. Male Sprague–Dawley rats (150–200 g) were anaesthetized by intraperitoneal injection of pentobarbitone sodium (60 mg kg^{-1} body wt). The rat tail artery was removed and freed of connective tissue under a dissecting microscope. The animals were then killed with an overdose injection of pentobarbitone sodium. The arteries were cut open longitudinally and immersed in a Ca^{2+}- and Mg^{2+}-free solution (Hanks' buffered saline solution, HBSS, Gibco) for 1 h at 4 °C. The arterial strips were then processed in the following solutions at 37 °C: (1) low-Ca^{2+} (0·2 mM) HBSS containing collagenase/dispase (1·5 mg ml^{-1}, Boehringer Mannheim GmbH),

elastase (0·5 mg ml^{-1}, type II, Sigma), trypsin inhibitor (1 mg ml^{-1}, Sigma) and bovine serum albumin (BSA, 2 mg ml^{-1}, Sigma) for 1 h; (2) Ca^{2+}-free HBSS in which the tissue was rinsed twice; (3) Ca^{2+}-free HBSS with collagenase 1 mg ml^{-1} (type II, Sigma), trypsin inhibitor 0·3 mg ml^{-1} and BSA 2 mg ml^{-1} for 45 min. Next, the arterial pieces were transferred to Ca^{2+}-free HBSS at 4 °C and triturated for 5 min. The calcium concentration of the incubating solution was increased to 1·7 mM by the stepwise addition of $CaCl_2$. The dispersed cells were then plated onto separate Petri dishes with Dulbecco's modified Eagle's medium (DMEM, Gibco) containing insulin (0·8 U ml^{-1}, Sigma), penicillin (100 U ml^{-1}, Sigma), and streptomycin (0·1 mg ml^{-1}, Sigma) and maintained at 4 °C for 4 h. Finally, fetal calf serum (Gibco) was added to the medium to reach a concentration of 10% and the cells were cultured at 37 °C. Cells were used within 18–30 h of isolation.

Inward current recording

The standard gigaohm seal, whole-cell version of the patch-clamp technique was used to measure whole-cell inward currents (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). The Petri dish with attached cells was mounted on the stage of an inverted phase-contrast microscope (Nikon, Japan). The pipette (internal) solution contained (mM): caesium aspartate, 75; EGTA, 10; ATP, 2; $MgCl_2$, 5; potassium pyruvate, 5; potassium succinate, 5; glucose, 25; HEPES, 15; creatinphosphate-sodium, 5; creatin-kinase, 50 U ml^{-1}; (pH 7·4 with CsOH). The bath solution was of the following composition (mM): $BaCl_2$, 20; glucose, 10; Tris, 105; tetrodotoxin (TTX), $0{\cdot}5 \times 10^{-3}$, HEPES, 20; KCl, 5; CsCl, 5; glucose, 20; (pH 7·4 with HCl). Barium was used as the inward charge carrier. The pipette and bath solutions were chosen to isolate the barium current from the potassium current. Although sodium channels are absent or at a very low density in this cell preparation (Wang *et al.* 1989), the use of TTX in the Na^+-free bath solution effectively eliminated any possible sodium current. The osmolality of all solutions was adjusted to 310–330 mosmol. Currents were filtered with a 4-pole Bessel filter at a cut-off frequency of 5 kHz. The current traces were digitized using a personal computer system. The interface was a data translation DT2801A driven by VCAN (software package provided by Dr J. Dempster, University Strathclyde, Glasgow). Leak and capacitative currents were subtracted with the use of Axopatch-1B electronics or VCAN software. In most cases, series resistance was compensated (Axopatch-1B, Axon Instruments). Since all the cells used were spherical or oval in shape with a diameter of approximately 24 μm or dimensions of 20×15 μm, an optimal space clamp was usually obtained. During the process of forming a gigaohm seal between the tip of the pipette and the patch of the membrane, some cells contracted to such a degree that a gigaohm seal could not be achieved. Data were analysed using VCAN. In all cases, the peak current (leak current corrected) was used to construct the current *vs.* voltage (I–V) relationships.

In a previous publication (Wang *et al.* 1989), the decline of inward currents with time ('run-down') was described. Immediately after the cell was voltage clamped, the inward current increased, possibly as the result of the outward K^+ current being blocked by a high concentration of Cs^+ in the pipette and Ba^{2+} in the bath (Armstrong & Taylor, 1980; Quandt & Narahashi, 1984). A relatively stable period of barium currents followed. If the current magnitude did not change from the third to the fifth minute after the rupture of the cell membrane, the rate of 'run-down' of the inward current was usually negligible for 20 min or longer (Wang *et al.* 1989). Cells which had stable inward currents from the third to fifth minute after penetration of the membrane were used. If the current decayed too quickly, it was difficult to distinguish 'run-down' of the current from the inhibitory or excitatory effect of the agents. Cells which had a fast decline of inward currents within this period were discarded. At the end of the fifth minute, inward barium currents were recorded and the I–V relationship was plotted as the control. Different fragments of bovine PTH (bPTH) were then added to the bath and inward barium currents were measured again (usually within 5 min of drug administration). In some cells the bath solution was changed to wash PTH preparations out in order to determine whether the PTH effect is reversible. This procedure further excluded the influence of current 'run-down'. Therefore, no allowance was made for the spontaneous decline of the inward current in measuring the effect of PTH on the magnitude of inward barium currents. When the recorded amplitude of inward currents was 5% higher or lower than that obtained at the previous recording (30 s interval), an increase or decrease, respectively,in the amplitude of the inward current was defined. In an attempt to investigate the specificity of the effect of PTH on L-channel currents, Bay K 8644, an L-channel agonist, was applied before or after the application of bPTH-(1–34) to the same cell in some cases.

Unless otherwise specified, a 200 ms test pulse to different membrane potentials was applied every 10 s. The experiments were carried out at room temperature (20–23 °C).

Drugs

PTH fragments, including bPTH-(1–34), bPTH-(3–34) and bPTH-(7–34), were purchased from Bachem Inc. They were dissolved in distilled water to make a $2{\cdot}43 \times 10^{-4}$ M stock solution and stored at −80 °C. Bay K 8644 (Calbiochem) was dissolved in absolute ethanol to make a 2×10^{-3} M stock solution. Nifedipine (Sigma) was dissolved in acetone and $LaCl_3$ (Sigma) was dissolved in distilled water. The final concentration of solvents, ethanol (0·27%) and acetone (0·3%), when added to the bath solution had no detectable effect on the magnitude or kinetics of the inward current. All the stock solutions of drugs were directly added to 3 ml of bath solution to give the expected final concentration. The final concentration of bPTHs in the bath was achieved by a single addition in order to avoid possible desensitization of the cell to the peptide. Only one experiment was conducted per dish. The time needed for drugs to distribute evenly within the dish and produce a steady-state response was 1–2 min.

Statistics

Unless original traces or single experiments are shown, values are expressed as means ± S.E.M. In most cases, it is the peak inward current that is used for displays. The paired *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.

RESULTS

Characterization of two types of voltage-dependent Ca^{2+} channel currents

In the present study, typical long-lasting (L-channel) and transient (T-channel) inward currents were recorded in smooth muscle cells from the rat tail artery as reported before (Wang *et al.* 1989). With the holding potential set at −40 mV, *L*-channel currents were detected at a membrane potential of −20 mV and were maximal at +20 mV with an apparent reversal potential beyond +60 mV. These currents were activated quickly and inactivated very slowly with a half-time of inactivation of > 150 ms. With the holding potential at −80 mV, T-channel currents were activated at −50 mV and were maximal at −20 mV. These currents inactivated quickly and completely (Fig. 1). The peak amplitudes of both L- and T-channel currents in this study could be maintained without significant deterioration for up to 20 min (Wang *et al.* 1989).

It has been established that only the L-channel in smooth muscle cells is sensitive to dihydropyridines (see review by Bean, 1989). The effects of nifedipine and Bay K 8644, a calcium antagonist and an agonist, respectively, on the L-channel current were determined in the present study. Bay K 8644 at a final concentration of 5 μM increased and nifedipine (1 μM) decreased (not shown) the magnitude of this inward current. Their effects on the L-channel current were detectable at 30 s and became stable within 5 min after the drug had been added to the bath. In addition, Bay K 8644 shifted the *I–V* relationship of the L-channel current by 10 mV toward more negative potentials and increased the inactivation kinetics. On the contrary, T-channel currents in these cells were not sensitive to dihydropyridines (not shown).

The pharmacological sensitivity to dihydropyridines as well as the electrophysiological properties of the inward currents in the present study are consistent with the characteristics of classical L- and T-channel currents (Benham *et al.* 1987; Bean, 1989; Wang *et al.* 1989).

Effect of bPTH-(1–34) on the T-channel current

T-channel currents in rat tail artery smooth muscle cells are difficult to measure possibly due to a low channel density in this preparation. The coexistence of L-channels in the same smooth muscle cells often masked small T-channel currents

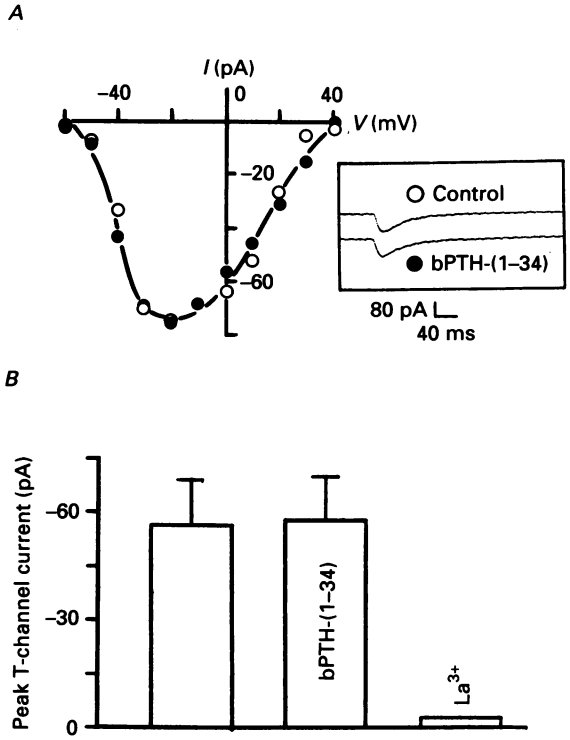

Fig. 1. The effect of bPTH-(1–34) on T-channel currents in vascular smooth muscle cells. These cells were pre-treated with nifedipine (5 μM) to block the L-channel current. *A*, the *I–V* relationship of the T-channel currents before (○) and after (●) the addition of 1 μM-bPTH-(1–34), respectively. The original current traces shown in the inset were taken from the same cell with the test pulse to −20 mV. The holding potential was set at −80 mV. *B*, the effect of bPTH-(1–34) on T-channel currents. Data from five cells was combined and shown as a bar graph. bPTH-(1–34) at a concentration of 1 μM did not change the peak amplitudes of T-channel currents. T-channel currents were eliminated by 2 mM-La^{3+}.

(Wang *et al.* 1989). Hence, in the present study, some cells were pre-treated with nifedipine at a concentration of 5 μM to block L-channels. Under this condition, relatively pure T-channel currents could be measured in some cases (holding potential of −80 mV). bPTH-(1–34) at a concentration of 1 μM had no effect on the peak amplitudes of relatively pure T-channel currents at all test potentials. This is shown in the current records and the *I–V* relationship (Fig. 1). The subsequent application of La^{3+} completely eliminated this transient inward current. Similar results were obtained in five cells.

Effect of bPTH-(1–34) on the L-channel current

The magnitude of L-channel currents was reduced in the presence of bPTH-(1–34). Usually, the decrease in the magnitude of L-channel currents induced by bPTH-(1–34) was evident at 2–3 min and reached a steady-state level within 5 min. In some

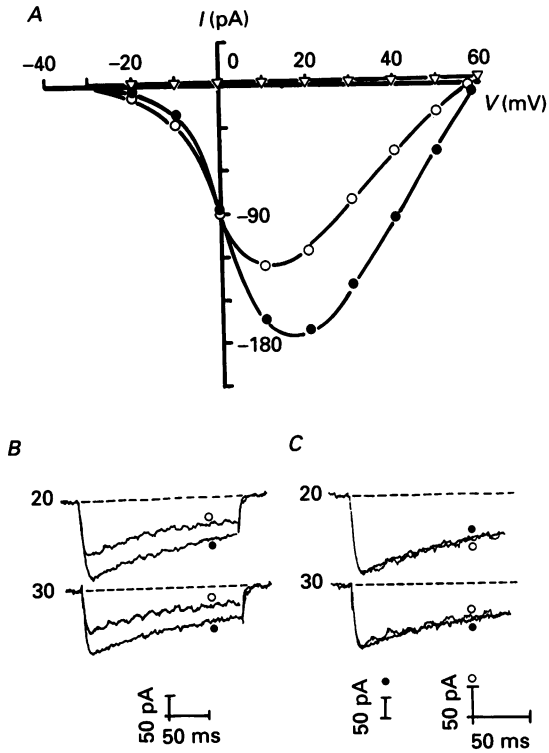

Fig. 2. The inhibitory effect of bPTH-(1–34) on L-channel currents in one smooth muscle from the rat rail artery. *A*, the *I–V* relationships under different experimental conditions. After stable currents were obtained as a control (●), 1 μM-bPTH-(1–34) was added to the bath for 5 min (○) and followed by 2 mM-La^{3+} (▽). The application of La^{3+} completely blocked the inward current, which is shown by the linear *I–V* relationship. *B*, the original current traces before and after the addition of bPTH-(1–34). Test potentials shown are in mV. *C*, The current traces in the presence of bPTH-(1–34) were re-scaled and superimposed on the control current traces using the same scale as in *B*. The superimposed currents showed that bPTH-(1–34) did not change the kinetics of inactivation of L-channel currents. Leak and capacitative currents have been subtracted. Holding potential, −40 mV.

cells ($n = 3$), after the initial 5 min decrease, the magnitude of L-channel currents recovered to the control level, suggesting the existence of a mechanism of desensitization to bPTH-(1–34) (data not shown). Figure 2 shows, in one cell, that 1 μM-bPTH-(1–34) inhibited the L-channel current by 26·3%. The effect was most evident at the peak of the *I–V* relationship. In fourteen cells, the maximum inward current was 74 ± 13 pA and 49 ± 10 pA before and after the application of 1 μM-

bPTH-(1–34), respectively ($P < 0.01$). The leak current and the current required to hold the cell membrane at −40 mV were not changed by the bPTH-(1–34) (not shown).

To observe the effects of bPTH-(1–34) on the kinetics of L-channel currents, the inward currents evoked from a holding potential of −40 mV in the absence or presence of bPTH-(1–34) were compared. When the reduced current records obtained in the presence of bPTH-(1–34) (Fig. 2*B*) were scaled and superimposed on the inward current trace before application of bPTH-(1–34), identical traces were obtained (Fig. 2*C*), indicating that bPTH-(1–34) has no effect on the kinetics of L-channel currents. In addition, the threshold potential for the activation of L-channel currents, the potential at which the maximum inward currents occurred and the zero current potential (apparent reversal potential) for calcium channel currents were not changed in the presence of bPTH-(1–34).

The same reversal potential for L-channel currents in the absence or presence of bPTH-(1–34) indicated that the reduction in L-channel currents by bPTH-(1–34) was not due to changes in the intracellular concentration of free calcium. In addition, the inhibition of L-channel currents in the present study is mainly due to the specific effect of bPTH-(1–34) on L-channel currents without the contamination of outward currents. The evidence for this is as follows: (1) the compositions of pipette solution (150 mM-Cs^+) and bath solution (20 mM-Ba^{2+}) were designed to eliminate the voltage-dependent K^+ current and the Ca^{2+}-dependent K^+ current (Quandt & Narahashi, 1984; Benham, Bolton, Lang & Takewaki, 1985); (2) application of 2 mM-La^{3+} revealed a linear relationship between voltage and current in the presence of bPTH-(1–34) over the range of voltages associated with K^+ current activation (Fig. 2*A*; Dunlap & Fischbach, 1981) and (3) the steady-state activation curve was not shifted by bPTH-(1–34), see Fig. 4*D*.

As suggested from many studies on the pharmacological responsiveness of voltage-dependent calcium channels, such as the effect of calcitonin gene-related peptide on N-type (neuron) calcium channels (Ryu, Gerber, Myrase & Randie, 1988), vasopressin on L-channels (Mollard, Vacher, Rogawski & Dufy, 1988) and noradrenaline on L-channels (Benham & Tsien, 1988), responsiveness of L-channels in rat tail artery cells to bPTH-(1–34) was not consistent in every case. In a total of forty-six cells, bPTH-(1–34) at different concentrations produced a decrease in L-channel currents in thirty-four cells, compared to the control. Since we only tested one cell in one dish, we cannot be certain if all cells in the same dish had the same reactivity to bPTH-(1–34). Some of the many factors which may be responsible for variability of the bPTH-(1–34) effect are considered in the Discussion.

Characteristics of the effect of bPTH-(1–34) on L-channel currents

The effect of bPTH-(1–34) is dose dependent

PTH lowered the blood pressure *in vivo* and inhibited the tension development in strips from rat tail artery *in vitro* in a dose-dependent manner (Pang *et al.* 1988). Whether the effect of PTH on the inward current in single smooth muscle cells could be related to the concentration used was examined in the present study. Figure 3 shows the dose-dependent inhibition of L-channel currents by bPTH-(1–34). At

10^{-7} M, bPTH-(1–34) decreased the peak inward currents to 81·1 ± 3 % of the control values in seven cells ($P < 0.05$). The threshold concentration for inhibitory action of bPTH-(1–34) is, therefore, $< 10^{-7}$ M. When the concentration of bPTH-(1–34) was raised to 10^{-5} M, 59·6 ± 6 % of the L-channel current still remained.

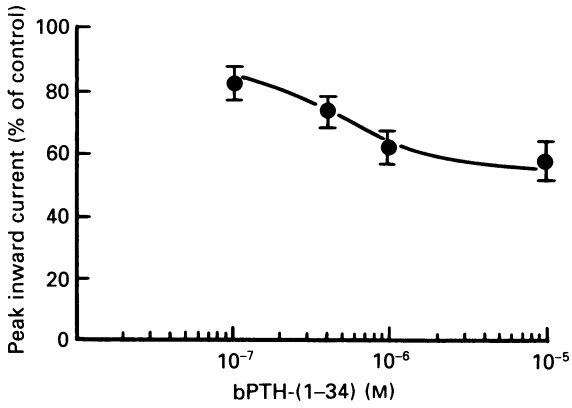

Fig. 3. Dose–response relationship of L-channel currents to various concentrations of bPTH-(1–34). The number of cells tested at each concentration ranged from 6 to 14. bPTH-(1–34) significantly reduced the inward currents at all concentrations tested ($P < 0.01$). Statistical significance was determined by analysis of variance in conjunction with the Newman–Keul's test. The difference is also significant among 0·1, 0·3 and 1 μM groups but not between 1 and 10 μM groups. Bars represent S.E.M.

The effect of bPTH-(1–34) is voltage dependent

It has been reported that, by changing the membrane potential, the inhibitory effect of some calcium antagonists on L-channel currents could be modified, such as in smooth muscle cells from rat mesenteric arteries (Bean, Sturek, Puga & Hermsmeyer, 1986) and neuroblastoma cells (Ogata, Yoshii & Narahashi, 1989). Hence, experiments were designed to determine the voltage dependence of the bPTH-(1–34) effect on L-channel currents in rat tail artery smooth muscle cells.

At first, the effect of bPTH-(1–34) at two different holding potentials, −60 and −20 mV, was investigated. Figure 4*A* shows the results from one smooth muscle cell. When the holding potential was set at −20 mV, the peak inward current was decreased by 34·5 % by a concentration of 1 μM-bPTH-(1–34). However, with the holding potential at −60 mV, the peak inward current was not depressed in the presence of bPTH-(1–34). This result suggested that bPTH-(1–34) inhibited L-channel currents more strongly at more positive holding potentials. Similar results were observed in three other cells.

This mechanism was examined in more detail by generating the full range of steady-state inactivation curves in the absence or presence of bPTH-(1–34). The holding potential was changed from −80 to +10 mV and the step command was applied to depolarize the membrane to +20 mV. The membrane was held for 15 s at each holding potential set. The steady-state inactivation curves were plotted using either the absolute value of the peak inward current as in Fig. 4*B* or the normalized

amplitude of the inward currents as in Fig. 4*C* against the holding potentials. The Boltzmann distribution of the form: $I = I_{max}[1+\exp(V-V_h)/k]^{-1}$ was used to fit the steady-state inactivation curves in Fig. 4*C*. In this equation, V represents the holding potential, V_h is the potential at which one-half of the calcium channels are

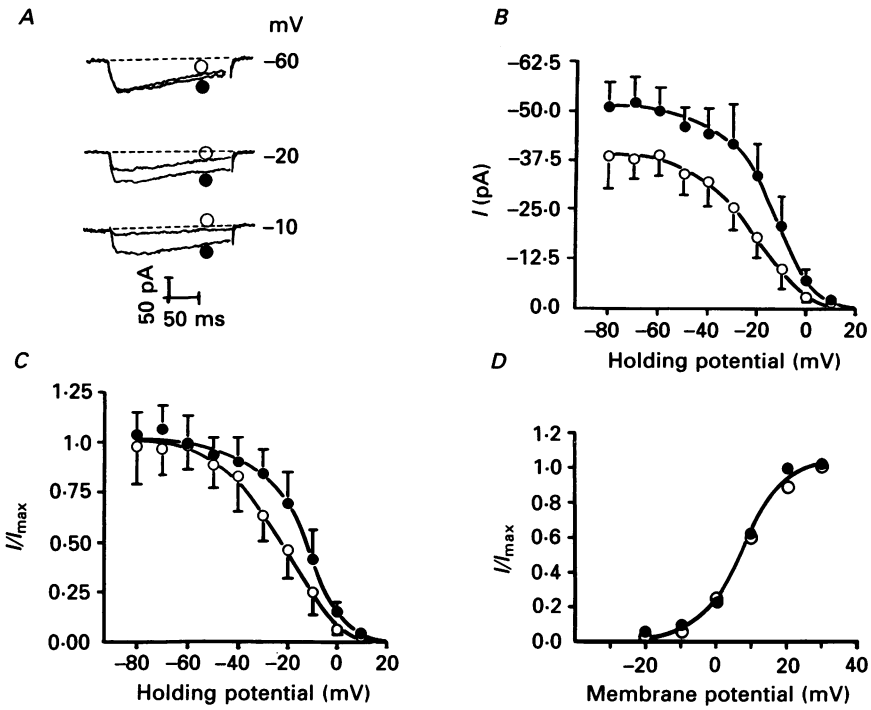

Fig. 4. The steady-state inactivation and activation curves of L-channel currents before (●) and after the addition of bPTH-(1–34) (○). *A*, currents were elicited from holding potentials of −60, −20 and −10 mV to a test potential of +30 mV before and after the addition of bPTH-(1–34) in one cell. Leak and capacitative currents have been subtracted. *B*, the steady-state inactivation of L-channel currents in the absence and presence of 1 μM-bPTH-(1–34). The data from six cells were combined and plotted as the means ± S.E.M. ($n = 6$). The peak current value shown in *A* was also included in *B*. The data shown in *B* were replotted in *C*, using the normalized amplitude of inward currents. The inactivation curves in *C* were least squares fitted to the equation: $I = I_{max}[1+\exp(V-V_h)/k]^{-1}$. Before the addition of bPTH-(1–34), $V_h = -14{\cdot}9$ mV and $k = 8{\cdot}7$ mV. After the addition of the peptide, $V_h = -24{\cdot}0$ mV and $k = 11{\cdot}5$ mV. At holding potentials from −30 to −10 mV the difference between the two curves is significant (Student's t test, $P < 0{\cdot}05$). *D*, the steady-state activation curves in the absence or presence of 1 μM-bPTH-(1–34) in one cell. The continuous line represents the least squares fit to the control data by the Boltzmann distribution of the form: $I = I_{max}[1+\exp(V_h-V)/k]^{-1}$ with $V_h = 7{\cdot}8$ mV and $k = 6$ mV.

inactivated and k is the slope factor. bPTH-(1–34) at 10^{-6} M decreased the amplitude of L-channel currents at all the holding potentials tested (Fig. 4*B*) but its effect was more potent at more positive holding potentials (Fig. 4*C*). When the holding potential was within the range of −30 to −10 mV, the inhibition of L-channel currents as well as the negative shift of the steady-state inactivation curve in the presence of

bPTH-(1–34) were more evident. It can be seen from Fig. 4*B* and *C* that bPTH-(1–34) shifted the steady-state inactivation curve to the left. The same phenomenon occurred in the presence of dihydropyridines, such as Bay K 8644 (Wang *et al.* 1989) and nicardipine (Terada, Kitamura & Kuriyama, 1987). The slopes of the relative inactivation curves (least squares fit to the experimental data) shown in Fig. 4*C* are 8·7 and 11·5 mV in the absence or presence of bPTH-(1–34), respectively. V_h changed from −14·9 to −24 mV before and after the application of the peptide, respectively.

To further examine the voltage dependence of the activation of L-channel currents, the steady-state activation curves in the absence or presence of bPTH-(1–34) were compared (Wang *et al.* 1989). These curves were plotted as relative peak amplitudes of L-channel currents against the test pulse, and fitted by the Boltzmann distribution of the form: $I = I_{max}\,[1+\exp(V_h - V)/k]^{-1}$, where V is the test potential which drives the inward current, V_h is the potential at which half-maximum inward current was obtained and k is the slope of the fitted curve. The steady-state activation of the L-channel current in this cell preparation was a steep function of voltage, and increased markedly over the potential range from 0 to 20 mV. Figure 4*D* shows that, in one cell, the activation of L-channel currents as a function of potential in the absence or presence of bPTH-(1–34) were identical. In another five cells examined, bPTH-(1–34) also produced no change either in the V_h values or the slope factors of the steady-state activation curves of L-channels.

The effect of bPTH-(1–34) is not dependent on the pulse lengths

All the experiments described above were executed with a 200 ms depolarization pulse. It may be argued that bPTH-(1–34) could bind more tightly to L-channels if the channel remained in the activated state for a longer period of time. To clarify this point, the depolarizing pulse was prolonged to 3 or 10 s. In the absence of bPTH-(1–34), there was no difference among the amplitude of L-channel currents with 200 ms, 3 s or 10 s pulse lengths. After application of bPTH-(1–34), the amplitude of L-channel currents decreased to the same extent with different pulse lengths. In addition, the duration of the interpulse interval, which was varied from 20 ms to 5 s, also did not influence the effect of bPTH-(1–34) on the amplitude of L-channel currents (data not shown).

The effect of bPTH-(1–34) on L-channel currents is reversible

After the inhibition of L-channel currents by bPTH-(1–34) was established, the cell was then perfused with PTH-free bath solution to check the reversibility of the effect of bPTH-(1–34). Satisfactory wash-out of bPTH-(1–34) was obtained with a perfusion rate of approximately 1 ml 10 s^{-1}. As shown in Fig. 5, the peak amplitude of the L-channel currents recovered to 93% of the control value after 2 min of wash-out and a 3 min recovery. Similar results were obtained when the wash-out experiments were conducted in four other cells.

The effect of bPTH-(1–34) on the tail currents of L-channels

Immediately following the end of a depolarization pulse, the calcium channels deactivated in response to repolarization to generate an instantaneous current or 'tail current'. The amplitude of tail currents reflects the instantaneous activity of the

channel and the time course represents the kinetics of the closing process(es) of calcium channels. In the present study, the tail currents mainly measured the deactivation of L-type calcium channels because a holding potential of −40 mV inactivated T-type calcium channels and a 40 ms pulse fully activated L-channels

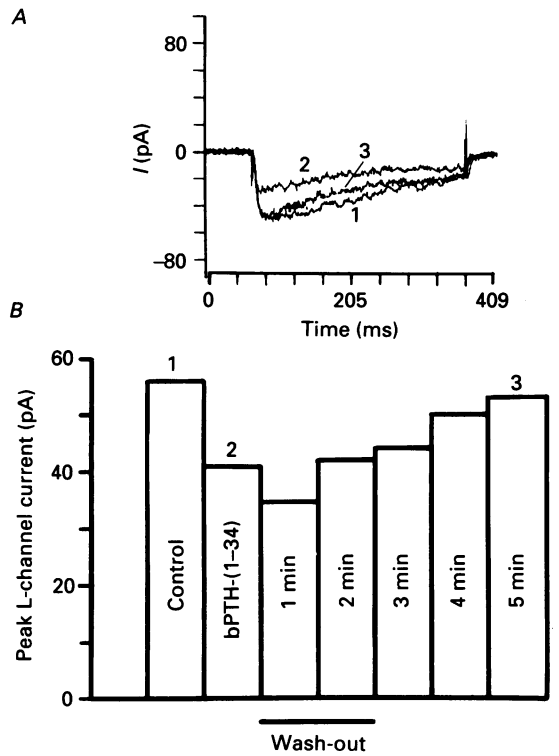

Fig. 5. The reversibility of bPTH-(1–34)-induced inhibition of L-channel currents. bPTH-(1–34) at a concentration of 1 μM inhibited the L-channel current by 73%. At the first minute of the wash-out, the peak amplitude of L-channel currents further decreased. The amplitude of L-channel currents then gradually recovered toward the pre-PTH level. The numbers attached to the original current traces (*A*) correspond to the different conditions indicated in the histogram, *B*. The data were pooled from the same cell.

but inactivated most T-channels. When the membrane was repolarized to −40 mV from different test pulses, the time course of the tail current decay was well fitted by a single exponential function (Fig. 6*A*), which is comparable to the tail current of L-channels in neuroblastoma cells (Yoshii, Tsunoo & Narahashi, 1988).

In view of this, we measured the instantaneous amplitude of the tail current at time zero, the instant at which the repolarization was initiated, by extrapolating the falling phase of the tail current with a single exponential function. The current–voltage relationships of the tail currents were constructed and are shown in Fig. 6*B* (square symbols).

Quite different from the bell shape of the *I–V* relationship obtained during the test pulse, the sigmoidal *I–V* plot could be determined for the instantaneous amplitude

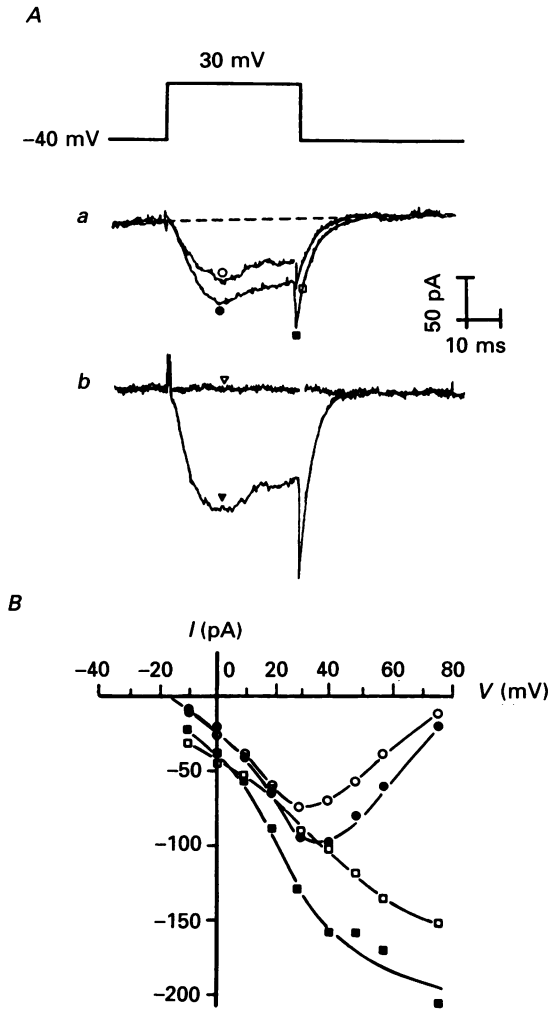

Fig. 6. The effect of bPTH-(1–34) on the deactivation of L-channel currents. *A*, tail currents generated from a holding potential of −40 mV to 30 mV. Records in (*A a* and *b*) are taken from the same cell. The depolarizing pulse was 40 ms. Leak and capacitative currents have been subtracted. The tail currents have been extrapolated to zero time at which repolarization was initiated using a single exponential function. Further explanation is found in the text. The tail current (control) had an instantaneous amplitude of 130 pA and a time constant of decay of 5·01 ms. Subsequent application of bPTH-(1–34) decreased the tail current to 92·8 pA with a time constant of 4·91 ms. Bay K 8644 (5 μM) reversed the inhibition of L-channel currents by bPTH-(1–34) (▼), with an instantaneous amplitude of tail current of 222 pA and a time constant of 3·69 ms. The final application of 2 mM-La^{3+} (▽) eliminated both the inward current during the test pulse and the tail current. *B*, the *I–V* relationships for both the peak inward current and the tail current, repolarized to −40 mV from step depolarizations in one cell. The application of bPTH-(1–34) decreased the instantaneous amplitude of tail currents which was proportional to the decrease in the peak inward current. In *A* and *B*, circles represent the peak inward current before (●) and after (○) the addition of 1 μM-bPTH-(1–34); squares represent the tail current before (■) and after (□) the addition of bPTH-(1–34).

of the tail current when repolarized to the holding potential from the step depolarizations. The amplitude of the tail current approached a plateau at +80 mV (Eckert & Douglas, 1983).

The change in the amplitude of the instantaneous tail current is proportional to that of the instantaneous calcium channel conductance. As expected, application of

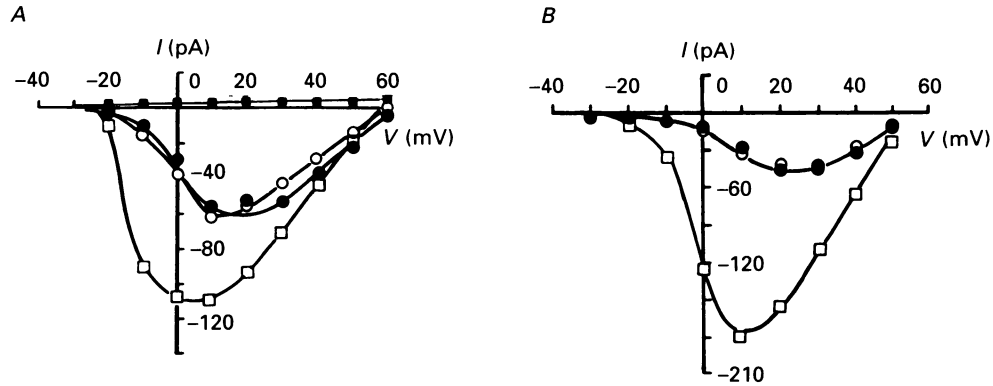

Fig. 7. The effect of bPTH-(3–34) on L-channel currents. *A*, bPTH-(3–34) alone did not affect L-channel currents. After a stable recording was obtained (●), 1 μM-bPTH-(3–34) was added to the bath (○). Five minutes later, 5 μM-Bay K 8644 was applied (□) and followed by 2 mM-La^{3+} (■). *B*, pre-treatment with bPTH-(3–34) abolished the inhibitory effect of bPTH-(1–34) on L-channel currents in one smooth muscle cell. The cell was pre-treated with 1 μM-bPTH-(3–34) for 15 min (●) and then exposed to 1 μM-bPTH-(1–34) (○). Under these conditions, bPTH-(1–34) did not change the L-channel current. Subsequent application of 5 μM-Bay K 8644 (□) increased the inward current. Two different cells are used in *A* and *B*, respectively. Holding potential, −40 mV. Leak and capacitative currents have been subtracted.

bPTH-(1–34) decreased the amplitude of the instantaneous tail current to the same degree as the decrease in the amplitude of peak inward current during the test pulse (Fig. 6*A*) in the presence of the peptide. However, the decay of the tail current upon repolarization was not affected by bPTH-(1–34) (Fig. 6*Aa*), suggesting that the gating mechanism controlling the closing process of L-channels was not altered by the peptide. The time constants of tail current after being depolarized to +30 mV were 5·22±0·5 ms in the absence and 5·60±0·4 ms in the presence of bPTH-(1–34) ($n = 5$).

Effect of bPTH-(3–34) and bPTH-(7–34) on L-channel currents

In an attempt to compare the effect of different fragments of bPTH on L-channel currents, bPTH-(3–34) and bPTH-(7–34) were used in the next group of experiments. When the time course of changes in the magnitude of L-channel currents in response to bPTH-(3–34) and bPTH-(7–34) was followed up to 5 min after these two fragments were added, neither a decrease nor increase in the amplitude of L-channel currents was seen. bPTH-(3–34) at a concentration of 10^{-6} M had no effect during the 5 min period of application. As shown in Fig. 7*A*, the *I–V* curves before and after application of bPTH-(3–34) were the same. Figure 8 shows neither 10^{-5} M-bPTH-

(7–34) in seven cells nor 10^{-6} M-bPTH-(3–34) in fifteen cells affected L-channel currents. No change in the kinetics of L-channel currents could be detected in the presence of bPTH-(3–34) or bPTH-(7–34).

Effect of pre-treatment of cells with bPTH-(3–34) on subsequent changes in L-channel currents induced by bPTH-(1–34)

To examine whether the change in L-channel currents induced by bPTH-(1–34) is exerted via the PTH specific receptor(s), we tested the effect of bPTH-(1–34) on the

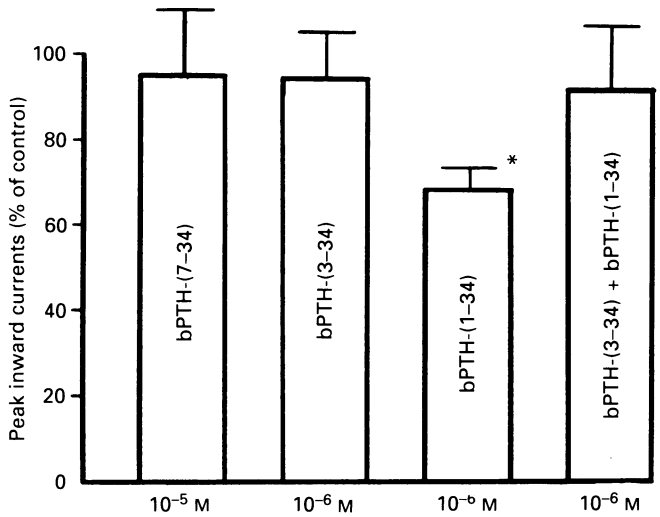

Fig. 8. Summary of the effect of different fragments of bPTH on L-channel currents. The effects of bPTH fragments were evaluated as the percentage change in L-channel currents of the control value. The concentrations of the peptide fragments are indicated beneath the bar graph. Only bPTH-(1–34) significantly inhibited L-channel currents and this inhibitory effect was abolished by pre-treatment of cells with bPTH-(3–34). *, $P < 0{\cdot}01$.

L-channel current after pre-treatment of cells with bPTH-(3–34), which is a PTH antagonist (Nickols, Metz & Cline, 1986). It was shown that bPTH-(3–34) did not modify L-channel currents by itself (Fig. 7*A*). However, when one cell, shown in Fig. 7*B*, was pre-treated with 10^{-6} M-bPTH-(3–34) for more than 10 min, the addition of 10^{-6} M-bPTH-(1–34) was ineffective in terms of the inhibition of L-channel currents. The data from this cell show that bPTH-(3–34) is capable of blocking the inhibitory effect of bPTH-(1–34) on L-channels. Results from more cells further supported this hypothesis (Fig. 8). In ten cells, the L-channel current had a magnitude of $40{\cdot}3 \pm 5{\cdot}0$ pA after pre-treatment of the cells with 10^{-6} M-bPTH-(3–34). Five minutes after 10^{-6} M-bPTH-(1–34) was added to the bath solution, the magnitude of L-channel currents was $36{\cdot}2 \pm 7$ pA. There was no significant difference in channel currents between these two treatments.

The absence of an inhibitory effect of bPTH-(1–34) on L-channel currents in smooth muscle cells may be explained if bPTH-(3–34) occupies the same binding sites as bPTH-(1–34) and thus decreased bPTH-(1–34) binding. It is also possible that bPTH-(3–34) binding changed the configuration of the L-channel complex in

such a way that the L-channels lost their pharmacological response to the subsequent application of any agent. If the latter were the case, the subsequent challenge to the L-channel by Bay K 8644 would be ineffective. However, the results shown in Fig. 7 indicated that Bay K 8644 was still able to enhance L-channel currents, suggesting a normal responsiveness of L-channel protein and the existence of different binding sites on or near L-channel proteins for PTH fragments and Bay K 8644.

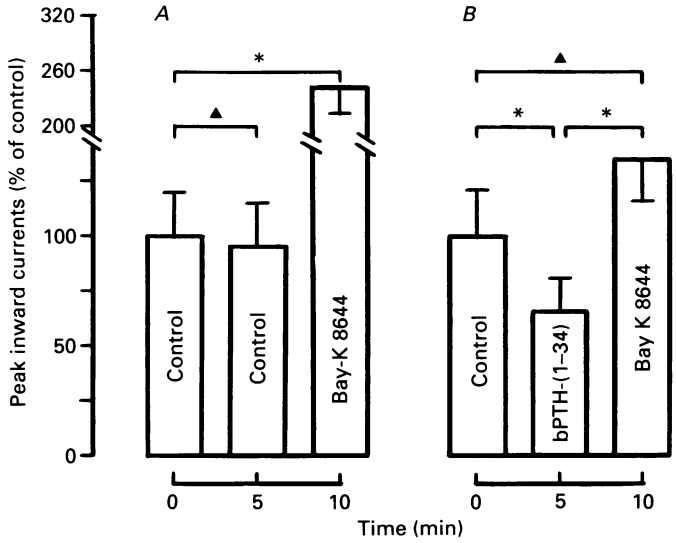

Fig. 9. The amplification of L-channel currents by Bay K 8644 in the absence or presence of bPTH-(1–34). *A*, the control value was measured first. Five minutes later the peak inward current was measured again, and then 5 μM-Bay K 8644 was applied. At 10 min the current amplitude was recorded ($n = 7$). *B*, the peak inward current was recorded as the control and then 1 μM-bPTH-(1–34) was added. At 5 min, the peak inward current in the presence of bPTH-(1–34) was measured and 5 μM-Bay K 8644 was added. The Bay K 8644-induced change in the current amplitude was recorded at 10 min ($n = 10$). *, $P < 0{\cdot}01$. ▲, $P > 0{\cdot}05$.

Interactions of bPTH-(1–34) and Bay K 8644

The specificity of the effect of bPTH-(1–34) on L-channel currents was further examined by investigating the interactions of bPTH-(1–34) and Bay K 8644. In some cells, Bay K 8644 at a final concentration of 5 μM was first added to the bath. The subsequent application of bPTH-(1–34) inhibited the increase in L-channel currents induced by Bay K 8644 (not shown). In the other group of cells, bPTH-(1–34) was first added to the bath and Bay K 8644 was subsequently applied. Figure 9 shows that Bay K 8644 increased L-channel currents which were previously inhibited by bPTH-(1–34). Further comparison of the amplification of L-channel currents by Bay K 8644 in the presence or absence of bPTH-(1–34) was made. Without bPTH-(1–34), 5 μM-Bay K 8644 enhanced the inward current to 252 ± 37 % of the control value. At the same concentration, Bay K 8644 still increased the amplitude of L-channel currents by 204 ± 29 % in the presence of 1 μM-bPTH-(1–34) ($P < 0{\cdot}05$). However, Bay K 8644 increased L-channel currents by only 35 ± 19 %

compared to the control before the addition of bPTH-(1–34). Furthermore, bPTH-(1–34) failed to modify the steady-state activation curve of L-channels (Fig. 4*D*) but Bay K 8644 shifted the curve toward the negative direction by 10 mV (Wang *et al.* 1989). These results suggest that Bay K 8644 cannot completely reverse the inhibitory effect of bPTH-(1–34) on L-channel currents. One possible reason is that Bay K 8644 acts only on those active L-channels which are not inhibited by bPTH-(1–34).

DISCUSSION

Significance of the present study

The mechanisms by which living organisms regulate the activity of voltage-dependent calcium channels are poorly understood. It is reasonable to postulate the existence of endogenous calcium channel modulators, both antagonists and agonists, which modulate calcium channels under physiological and pathophysiological conditions. It has been reported that endothelin, a peptide produced by vascular endothelial cells, activates L-type voltage-dependent calcium channels in vascular smooth muscle cells, either directly or indirectly (Goto, Kasuya, Matsuki, Takuwa, Kurihara, Ishikawa, Kimura, Yanagisawa & Masaki, 1989). In the present study, we report that the active N-terminal fragment of PTH, bPTH-(1–34), inhibits L-channel currents in single smooth muscle cells from the rat tail artery. The results from this study confirm, considerably amplify and extend preliminary results (Pang *et al.* 1990). The inhibitory effect of bPTH-(1–34) is dose dependent and potential dependent, but does not rely on the duration of the activation of calcium channels, at least within the range tested in the present study. PTH, therefore, is the first identified endogenous circulating hormone capable of inhibiting (modulating) voltage- and time-dependent calcium channels in vascular smooth muscle cells. These findings will lead to a more critical evaluation of the mechanism of PTH action. The methodology and protocols established in this study will also assist in the identification of other endogenous substances responsible for the regulation of voltage- and time-dependent calcium channels.

Correlation with in vivo and in vitro studies

As previously reported, PTH lowers blood pressure *in vivo*. The tension development in rat tail artery strips, *in vitro*, elicited by 60 mM-KCl is attenuated by PTH. This PTH-induced effect was reduced by the removal of extracellular Ca^{2+} and mimicked by calcium channel blockers, such as D600. The identification of two types of voltage-dependent calcium channels in rat tail artery cells opens the possibility that PTH exerts its vasoactive action, at least in part, by modulating the influx of calcium through these channels. Our results provide direct evidence to support the view that PTH could inhibit calcium channels in vascular smooth muscle cells. This was previously suggested by a tension and calcium flux study at the tissue level (Pang *et al.* 1988). The rat tail artery possesses contractile properties similar to those of small resistance vessels (Frost, Gerke & Frewin, 1976). The activation of L-type calcium channels in the smooth muscles cells from rat tail artery might be the key link between excitation and contraction of this cell preparation. Consequently, the inhibition of L-channels by bPTH-(1–34) blocks the access of extracellular calcium

to the intracellular space of the cell. We believe this is part of the mechanisms of PTH-induced vasodilatation (Pang *et al.* 1988, 1990). Since PTH has different roles in various target organs and calcium channels behave differently in different tissues (Bean *et al.* 1986), it is reasonable to suggest that PTH may regulate calcium channels in a somewhat different manner in other tissues.

It has been estimated that the normal circulating concentration of PTH is approximately 10^{-10} M (Fischer, Binswanger & Dietrich, 1974; Carnes, Anast & Forte, 1980). The threshold concentration of bPTH-(1–34) for relaxing the rat tail artery strips constricted by KCl *in vitro* was approximately 10^{-8} M (Pang *et al.* 1988). In the present study, the minimal concentration of bPTH-(1–34) required to inhibit L-channel currents in single smooth muscle cell was 10^{-7} M. In studies where single cells are used (patch clamp), enzymatic treatment may cause damage to membrane proteins. It has been reported that in cell dialysis experiments the concentrations used to block or mimic the hormonal responses were much higher than those used in biochemical studies (10^{-9}–10^{-6} M; Hescheler, Tang, Jastorff & Trautwein, 1987). Furthermore, the artificial extracellular (bath) and intracellular (pipette) solutions used facilitate the recording of calcium channel currents, but may decrease the channel response to hormones or neurotransmitters. For example, the inhibitory effects of dopamine and noradrenaline on calcium channel currents were enhanced by a low extracellular calcium concentration (2 mM) but abolished by a high extracellular calcium concentration (20 mM; Marchetti, Carbone & Lux, 1986). It should also be noted that a high concentration of EGTA was used in the calcium-free pipette solution. As a result, the intracellular free calcium concentration in the present study was estimated to be less than 10^{-9} M (Sada, Kojima & Sperelakis, 1988). The low concentration of intracellular calcium will reduce the calcium-induced inactivation of calcium channel currents, but the activity of some intracellular calcium-dependent enzymes, however, will be decreased. If the effect of bPTH-(1–34) on L-channel currents in vascular smooth muscle cells is mediated by some of these calcium-dependent second messengers, a higher concentration of bPTH-(1–34) would be required to produce the effect which would be slower in onset. Bearing these considerations in mind, the concentrations of bPTH-(1–34) used in the present study may be related to physiological concentrations of PTH *in vivo*. Further experiments are needed to explore the physiological and pathophysiological actions of PTH in the homeostatic control of the cardiovascular system.

Mechanisms of the inhibitory effect of bPTH-(1–34) on L-channel currents

Although the mediation of L-channel currents by bPTH-(1–34) is evident, we still do not known whether the suppression of L-channel currents is due to the direct action of bPTH-(1–34) *per se* on calcium channel protein or due to the activation of some cytoplasmic second messengers through the binding of PTH to its specific receptor(s) outside the calcium channel protein(s). It is not possible to distinguish between these two possibilities from our present data. Furthermore, it remains possible that, under *in vivo* conditions, PTH-induced modulation of calcium channel currents is secondary to PTH-induced changes in the membrane potential, which occur independently of changes in Ca^{2+} permeability (Koch, Blalock & Schonbrun, 1988). It should also be pointed out that PTH might interact with some transducer

macromolecules such as membrane GTP-binding protein (G-proteins) and indirectly inhibit L-channel currents. Unfortunately, very little is known with respect to the role of G-proteins in the modulation of voltage-dependent calcium channels in vascular smooth muscle cells (Zeng, Benishin & Pang, 1989).

The phosphorylation–dephosphorylation mechanisms in the regulation of voltage-dependent calcium channels have received increasing attention. The involvement of the cyclic AMP-mediated pathway has been documented to be responsible for the activation of voltage-dependent calcium channels in normal rat anterior pituitary cells (Schofl, Meier, Gotz & Knepel, 1989). The bPTH-(1–34)-induced inhibition of L-channel currents may be mediated by cyclic AMP, since cyclic AMP is a potent relaxant for many types of smooth muscle (Hardman, 1981), and may mediate the hypotensive action of bPTH-(1–34) as a second messenger (Helwig, Schleiffer, Judes & Gairard, 1984). bPTH-(1–34) increased cyclic AMP content significantly in rat tail artery tissues and the vasodilatory action of bPTH(1–34) was potentiated by isobutylmethylxanthine, a phosphodiesterase inhibitor, and decreased by imidazole, a phosphodiesterase stimulator (Pang, Yang, Tenner, Kenny & Cooper, 1986). In cultured vascular smooth muscle cells, 1 min treatment with PTH resulted in 5- to 10-fold increases in intracellular cyclic AMP concentrations (Nickols, 1985). Preliminary results also indicated that the effect of bPTH-(1–34) on L-channel currents in rat tail artery was inhibited by Rp-cyclic AMPs, a cyclic AMP antagonist (R. Wang, E. Karpinski & P. K. T. Pang, unpublished observation). Cyclic AMPs is an analog of cAMP in which one of the two exocyclic oxygen atoms in the cyclic phosphate moiety is replaced by sulphur. Equatorial thio substitution produces the R-isomer. Detailed studies to correlate the effect of PTH on voltage-dependent calcium channels with the intracellular cyclic AMP level will be necessary to elucidate this possible mechanism.

In the present study, the interaction of bPTH-(1–34) and Bay K 8644, a calcium agonist, was also investigated. There are several reasons which justify the investigation of interactions of bPTH-(1–34) and Bay K 8644. Bay K 8644 is a specific L-channel agonist, and thus can be used to examine the channel specific effect of other L-channel modulators. The study of calcium channel modulation by β-adrenergic agents and Bay K 8644 has led to a critical understanding of the mechanisms of both agents (Tsien, Bean, Hess, Lansmann & Nowycky, 1986). This comparison is also important for the understanding of the mechanisms of bPTH-(1–34). In addition, Armstrong & Eckert (1987) demonstrated that Bay K 8644 modulated the gating of calcium channels only when the channel was phosphorylated. If the dephosphorylation of calcium channel currents was complete, the subsequent application of Bay K 8644 failed to modulate calcium channels. Hence, these authors suggested that the effect of Bay K 8644 on calcium channel currents depended on, and was influenced by, the status of phosphorylation–dephosphorylation of calcium channels. If the inhibitory effect of bPTH-(1–34) in smooth muscle cells was due to the dephosphorylation of L-channels, the ensuing application of Bay K 8644 would not be able to increase L-channel currents. Taken together, the elucidation of the possible interaction between the effects of bPTH-(1–34) and dihydropyridine agonists on L-channels will help in understanding the mechanisms of channel modulation under physiological conditions and pharmacological inter-

vention. In fact, the interaction of bPTH-(1–34) and Bay K 8644 on calcium channels in renal epithelial cells has been reported (Bacskai & Friedman, 1990). Our data demonstrated that Bay K 8644 increased L-channel currents which were previously inhibited by bPTH-(1–34). On the other hand, bPTH-(1–34) inhibited L-channel currents which were previously increased by Bay K 8644. In the presence of bPTH-(3–34), the effect of bPTH-(1–34) on L-channel currents was abolished, but the effect of Bay K 8644 still remained. In addition, our preliminary experiments have shown that PTH effects on L-channel currents in vascular smooth muscle cells are inhibited with treatment by Rp-cyclic AMPs, a cyclic AMP antagonist. However, the effect of Bay K 8644 could not be antagonized by Rp-cyclic AMPs treatment (R. Wang, E. Karpinski & P. K. T. Pang, unpublished observation). Hence, it does not appear to be possible that bPTH-(1–34) and Bay K 8644 could act on the same intracellular second messengers. Taken together, these results strongly suggest that bPTH-(1–34) and Bay K 8644 do not competitively bind to the same sites on cell membrane or act through the same signal transduction pathways. Bay K 8644 may augment L-channel currents by acting on a proportion of channels which are not dephosphorylated in the presence of bPTH-(1–34).

The antagonistic action of bPTH-(3–34) to bPTH-(1–34)

PTH is a charged protein, and is, therefore, not lipid soluble. It might only bind to specific receptors on the external side of the membrane and then modify the calcium channel. This hypothesis can be demonstrated, at least in part, by the antagonization of the effect of bPTH-(1–34) by bPTH-(3–34) which had no effect on L-channel currents by itself. Whether PTH-(3–34) and PTH-(1–34) bind to the same receptors in vascular smooth muscle cells is not clear at the present time. Nevertheless, it has been reported that bPTH-(1–34) and bPTH-(3–34) have approximately the same binding affinity to submitochondrial particles from bovine kidney cortex (Laethem & Zull, 1990). Furthermore, bPTH-(1–34) and bPTH-(3–34) are equipotent in eliciting an increase in extracellular calcium influx in rat osteoblast-like cells (Löwik, Van Leeuwen, Van der Meer, Van Zeeland, Scheven & Herrmann-Erlee, 1985). In bovine middle cerebral arteries, an equal molar concentration of bPTH-(3–34) could block approximately half the vasorelaxant effect of bPTH-(1–34) (Suzuki, Lederis, Huang, LeBlanc & Rorstad, 1983). Nickols & Cline (1987) reported that a fifty times higher concentration of bPTH-(3–34) was required to abolish bPTH-(1–34)-induced relaxation in rabbit aortic strips. In our study, the same concentration of bPTH-(3–34) and bPTH-(1–34) (10^{-6} M) was used. A concentration of 10^{-6} M-bPTH-(3–34) was fully effective in producing the complete inhibition of 10^{-6} M-bPTH-(1–34). This phenomenon can be explained if the binding sites for bPTH-(3–34) on the membrane, or the secondary events induced by bPTH-(3–34) inside the membrane, are saturated at a concentration of 10^{-6} M-bPTH-(3–34). Alternatively, bPTH-(3–34) at the same concentration as bPTH-(1–34) can completely abolish the effect of bPTH-(1–34) on voltage-dependent calcium channel currents in a single cell. However, the tension development in arterial strips described above was induced by prostaglandin $E_{2\alpha}$ ($PGE_{2\alpha}$) (Suzuki *et al.* 1983) or noradrenaline (Nickols & Cline, 1987). It would be more reasonable to suggest that the tension development in these reports was triggered by the intracellular release of

free Ca^{2+} or the activation of receptor-operated calcium channels in addition to the opening of voltage-dependent calcium channels. It is also important to recognize that the contraction of vascular smooth muscle is controlled by multiple factors; not only the extracellular calcium influx but also the intracellular calcium release (see review by Van Breemen & Saida, 1989) and phosphorylation of the myosin light chain by a Ca^{2+}–calmodulin-dependent myosin light chain kinase (see review by Kamm & Stull, 1989). Given different mechanisms underlying excitation–contraction coupling in smooth muscles, the complete inhibition of the effect of bPTH-(1–34) on L-type voltage-dependent calcium channel currents by bPTH-(3–34) at an equal molar concentration would be acceptable.

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