Calyculin A increases voltage-dependent inward current in smooth muscle cells isolated from guinea pig taenia coli

T. Usuki[a], K. Obara[a,*], T. Someya[a], H. Ozaki[b], H. Karaki[b], N. Fusetani[c] and H. Yabu[a]

[a] *Department of Physiology, Sapporo Medical College, W-17,S-1, Chuo-ku, Sapporo 060,* [b] *Department of Veterinary Pharmacology, and* [c] *Department of Marine Biochemistry, Faculty of Agriculture, University of Tokyo, Tokyo 113 (Japan)*

Received 3 December 1990; accepted 7 March 1991

Abstract. The effects of a potent phosphatase inhibitor, calyculin A (CL-A), on inward currents in guinea pig taenia coli smooth muscle cells were examined. CL-A increased the inward current, and this effect of CL-A was inhibited by a protein kinase C inhibitor, H-7, and by nifedipine. Phorbol 12,13-dibutyrate, an activator of protein kinase C, also increased the inward current and this effect was antagonized by H-7. These results suggest that in guinea pig taenia coli smooth muscle cells CL-A may facilitate the opening of the L-type Ca^{2+} channels through the protein kinase C-dependent phosphorylation system.

Key words. Calyculin A; phorbol ester; patch clamp technique; voltage-dependent Ca^{2+} channel; smooth muscle cell.

Calyculin A (CL-A) is one of the cytotoxic compounds isolated from an extract of marine sponges of the genus *Discodermia*[1]. CL-A is a potent phosphoprotein phosphatase inhibitor[2]. CL-A increased cytosolic free calcium concentration ($[Ca^{2+}]_i$) in rat aorta[3]. This increase in $[Ca^{2+}]_i$ was inhibited by verapamil, a Ca^{2+} channel blocker, or by removal of extracellular Ca^{2+}, and was potentiated by Bay K 8644, a 1,4-dihydropyridine (DHP) Ca^{2+} channel agonist. These observations led the authors to conclude that CL-A activated the voltage-dependent Ca^{2+} channel in smooth muscle.

Recently, direct measurements of Ca^{2+} channel currents in smooth muscles have been greatly facilitated by the development of patch-clamp techniques and of preparations of single smooth muscle cells[4-7].

In this study, we investigated the effect of CL-A on the inward current carried by Ba^{2+} through the voltage-dependent Ca^{2+} channel in guinea pig taenia coli smooth muscle cells.

Materials and methods

Single smooth muscle cells were isolated from guinea pig taenia coli by the method of Obara[8]. The taenia coli were incubated with 0.3% collagenase (type 1, Sigma), 0.6% trypsin inhibitor (type 1s, Sigma) and 1.0% bovine albumin in Ca^{2+}-free physiological salt solution (PSS) containing 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 5.6 mM glucose, and 4.2 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7.4, for 30 min at 35 °C. The freshly isolated cells were then incubated in modified KB medium[9] containing 110 mM KOH, 70 mM glutamic acid, 10 mM taurine, 25 mM KCl, 10 mM KH_2PO_4, 5 mM HEPES, 0.5 mM ethyleneglycol-bis(β-aminoethylether)-*N,N'*-tetraacetic acid (EGTA) and 11 mM glucose, pH 7.4, at 4 °C for 2–6 h before use.

Whole-cell currents were recorded under voltage clamp conditions similar to those described by Hamill et al.[10]. Ca^{2+} channel currents were isolated by the suppression of potassium currents using patch pipettes filled with 140 mM CsCl, 10 mM glucose, 4 mM EGTA, 5 mM HEPES and 4 mM Na_2ATP (pH 7.3). Inactivation of Ca^{2+} currents in guinea pig taenia coli smooth muscle cells is partially mediated by Ca^{2+} entry into smooth muscle cells[11]. To decrease Ca^{2+}-mediated inactivation of the Ca^{2+} current, we recorded inward currents carried by Ba^{2+}. The bath solution used for whole-cell recordings contained 135 mM NaCl, 5.4 mM KCl, 1.8 mM $BaCl_2$, 1 mM $MgCl_2$ and 5 mM HEPES (pH 7.4). During the experiment cells adhered to the glass bottom of a small chamber (volume about 0.7 ml) which was continuously perfused with the bath solution (flow rate about 1 ml/min). Drugs were applied by adding them in different concentrations to the perfusing solution. Experiments were carried out at room temperature (22–28 °C). CL-A was isolated from *Discodermia calyx*[1]. A stock solution was prepared by dissolving CL-A in ethanol, and a 1000-fold dilution of this solution was used for the experiments. Ethanol (0.1%) has no effect on the voltage-dependent Ca^{2+} channel currents of single smooth muscle cells isolated from guinea pig taenia coli.

Results

Figure 1 shows the effect of CL-A on inward currents carried by Ba^{2+} through voltage-dependent Ca^{2+} channels. Cells were depolarized with test potentials ranging from −70 mV to +40 mV from a holding potential of −80 mV. The inward current was resolvable when cells were depolarized positively to −40 mV and reached a maximum with a test potential of +10 mV. CL-A (10 nM) increased the peak inward current by an average of 21.2 ± 3.9% (means ± SE, n = 3). CL-A did not appear to affect the threshold for the activation of the inward current or the reversal potential obtained by extrapolation (control: +51.9 ± 2.4 mV, CL-A: +52.2 ± 2.2 mV; mean ± SE, n = 4). CL-A also had no effect on the voltage at which the maximum inward current was recorded. The effects of CL-A were dose-depen-

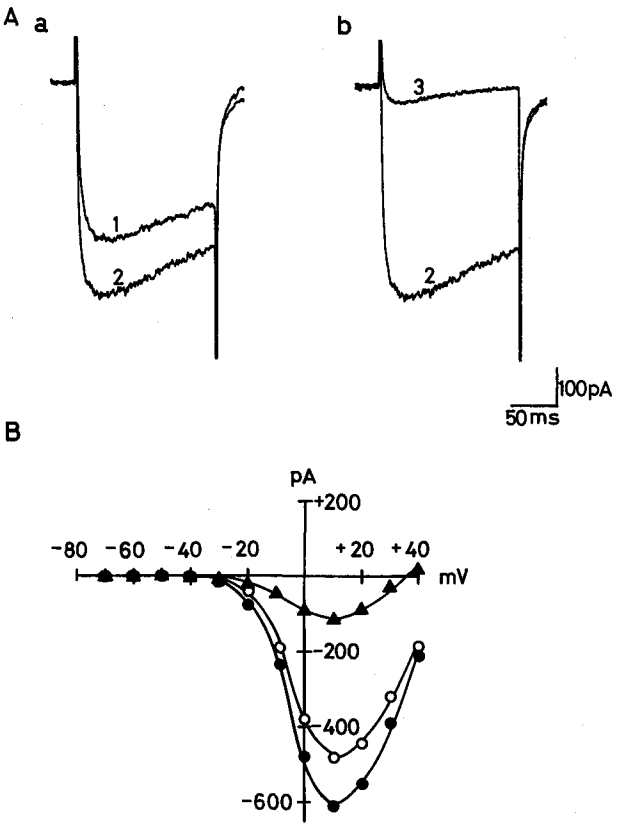

Figure 1. Effect of CL-A on the voltage-dependent inward currents. The drugs were applied for 3 min prior to depolarization. *A* Inward current elicited by a depolarizing step from holding potential of −80 mV to +10 mV in the absence (1) and the presence of 10 nM CL-A (2) or of 10 nM CL-A and 200 nM nifedipine (3). a) effect of CL-A. b) effect of CL-A and nifedipine. *B* Peak current-voltage relations for the cell in the absence (○) and the presence of 10 nM CL-A (●) or of 10 nM CL-A and 200 nM nifedipine (▲). Leak currents were subtracted.

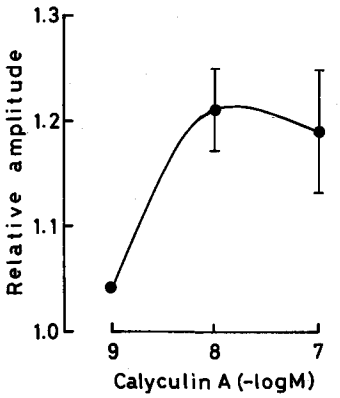

Figure 2. Effect of various concentrations of CL-A on inward current elicited by depolarization to +10 mV from a holding potential of −80 mV. CL-A was applied for 3 min prior to depolarization. The peak currents in the presence of CL-A were normalized to the value in the absence of CL-A. Each point represents mean ± SE from three experiments.

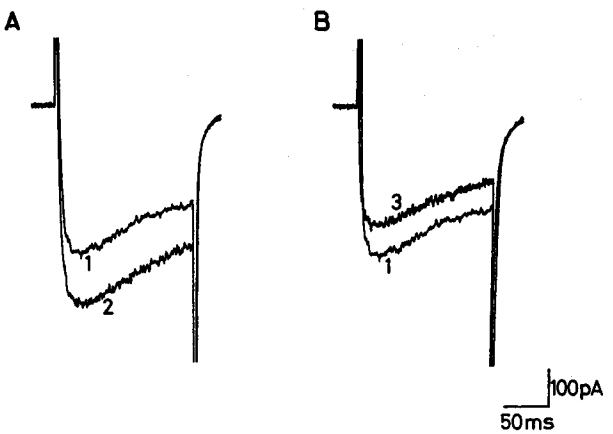

Figure 3. Effects of CL-A and H-7 on the voltage-dependent inward currents. The drugs were applied for 3 min prior to depolarization. Inward current was elicited by a depolarizing step from a holding potential of −80 mV to +10 mV in the absence (1) and the presence of 10 nM CL-A (2) or of 10 nM CL-A and 100 μM H-7 (3). *A* Effect of CL-A. *B* Effect of CL-A and H-7.

dent, and the maximum response was observed at about 10 nM CL-A (fig. 2). Simultaneous application of CL-A and nifedipine (200 nM), a DHP Ca^{2+} antagonist, decreased the inward current by about 90% of the control amplitude, which was almost equal to the amplitude decrease brought about by nifedipine alone. In the presence of CL-A, an additional application of 100 μM 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H-7), a protein kinase C inhibitor, caused a decrease in the inward current by 15.0 ± 5.0% (mean ± SE, n = 3) of the control amplitude which was almost equal to the amplitude decrease due to H-7 alone (fig. 3).

Figure 4 shows the effect of phorbol ester on inward currents. Phorbol 12, 13-dibutyrate (PDB, 100 nM), which is an activator of protein kinase C, increased the inward current by 27.5 ± 5.4% (mean ± SE, n = 3). In the presence of PDB, an additional application of 100 μM H-7 caused a decrease in the inward current to the control level. On the other hand, 4α-phorbol 12, 13-didecanoate (4α-PDD, 100 nM), which does not activate protein kinase C, had no effect on the inward current (101.1 ± 1.5%; mean ± SE, n = 3).

Discussion

The data show that CL-A increases voltage-dependent inward currents carried by Ba^{2+} through dihydropyridine (DHP)-sensitive Ca^{2+} channels in guinea pig taenia coli smooth muscle cells, in a concentration-dependent manner (fig. 2). The current that was enhanced by CL-A was blocked by nifedipine, a DHP Ca^{2+} antagonist (fig. 1). These results suggest that CL-A may facilitate the opening of the DHP-sensitive Ca^{2+} channels in these cells.

Recent patch clamp studies have provided evidence that multiple types of voltage-dependent Ca^{2+} channel exist in many excitable cells, including smooth muscle cells [5, 6, 12, 13]. Yoshino et al. [14] reported that guinea pig taenia coli smooth muscle cells contained at least two types of voltage-dependent Ca^{2+} channel (T- and L-

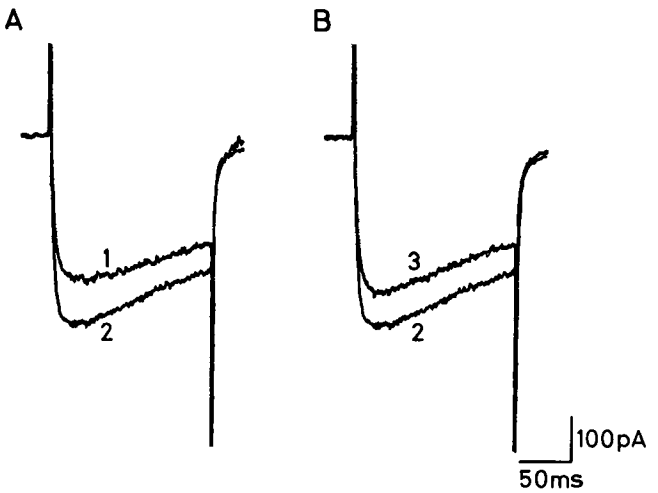

Figure 4. Effects of phorbol ester and H-7 on the voltage-dependent inward currents. The drugs were applied for 3 min prior to depolarization. Inward current was elicited by a depolarizing step from a holding potential of −80 mV to +10 mV in the absence (1) and the presence of 100 nM PDB (2) or of 100 nM PDB and 100 μM H-7 (3). *A* Effect of PDB. *B* Effect of PDB and H-7.

types), which have also been identified in other kinds of cell [12, 13]. The inward current in whole-cell recording was very sensitive to nifedipine (fig. 1). The DHP Ca^{2+} antagonist selectively inhibited the Ca^{2+} channel currents passing through the L-type voltage-dependent Ca^{2+} channels (L-current) [12, 13]. Therefore, it is suggested that the L-current is the predominant current component in whole-cell recording in guinea pig taenia coli smooth muscle cells.

In these cells, CL-A increased the inward current passing through the L-type voltage-dependent Ca^{2+} channel (figs 1 and 2). The opening of the L-type Ca^{2+} channel is suggested to be modulated by a phosphorylation of the L-type Ca^{2+} channel proteins by cAMP-dependent protein kinase (A-kinase) or Ca^{2+}/phospholipid-dependent protein kinase (protein kinase C) in cardiac [15] and smooth muscle cells [16, 17]. According to the current theory, the state of phosphorylation of protein is determined by the activity balance of protein kinase and protein phosphatase. Ishihara et al. [2] reported that CL-A had a potent inhibitory action on protein phosphatase activities. These observations suggest the possibility that CL-A produces its increasing effect on the inward current through suppression of the dephosphorylation process, resulting in an increased phosphorylation of protein (s) related to the calcium channel, which in turn facilitates the opening of Ca^{2+} channels.

Recently, Yabu et al. [18] suggested that in guinea pig taenia coli smooth muscle cells the effect of CL-A on the Ca^{2+} channel currents might not involve cAMP-dependent regulation of the L-type Ca^{2+} channels. However, the CL-A effect on the inward currents was inhibited by H-7, a protein kinase C inhibitor [19] (fig. 3). PDB, which is an activator of protein kinase C [20], increased the inward currents and this increase in the inward currents was inhibited by H-7 (fig. 4). Furthermore, 4α-PDD, which does not activate protein kinase C, had no effect on the inward currents. Therefore, the present results suggest that the effect of CL-A on the inward current does involve protein kinase C-dependent regulation on the L-type voltage-dependent Ca^{2+} channel.

In conclusion, in guinea pig taenia coli smooth muscle cells CL-A facilitates the opening of the L-type voltage-dependent Ca^{2+} channels, and the effect of CL-A on the inward current may involve protein kinase C activity.

Acknowledgments. We are grateful to Dr K. Sanders, Department of Physiology, University of Nevada, for helpful suggestions. This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

* To whom correspondence should be addressed.

1 Kato, Y., Fusetani, N., Matsunaga, S., and Hashimoto, K., J. Am. chem. Soc. *108* (1986) 2780.
2 Ishihara, H., Martin, B. L., Brautigan, D. L., Karaki, H., Ozaki, H., Kato, Y., Fusetani, N., Watabe, S., Hashimoto, K., Uemura, D., and Hartshorne, D. J., Biochem. biophys. Res. Commun. *159* (1989) 871.
3 Ishihara, H., Ozaki, H., Sato, K., Hori, M., Karaki, H., Watabe, S., Kato, Y., Fusetani, N., Hashimoto, K., Uemura, D., and Hartshorne, D. J., J. Pharmac. exp. Ther. *250* (1989) 388.
4 Yoshino, M., and Yabu, H., Pflügers Arch. *404* (1985) 285.
5 Worley, III, J. F., Deitmer, J. W., and Nelson, M. T., Proc. natl Acad. Sci. USA *83* (1986) 5746.
6 Benham, C. D., Hess, P., and Tsien, R. W., Circ. Res. *61* (1987) 10.
7 Bean, B. P., Sturek, M., Puga, A., and Hermsmeyer, K., Circ. Res. *59* (1986) 229.
8 Obara, K., Jpn. J. Physiol. *34* (1984) 41.
9 Isenberg, G., and Klockner, U., Pflügers Arch. *395* (1982) 6.
10 Hamill, O. P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F. J., Pflügers Arch. *391* (1981) 85.
11 Ganitkevich, V. Ya., Shuba, M. F., and Smirnov, S. V., J. Physiol. *392* (1987) 431.
12 Nowycky, M. C., Fox, A. P., and Tsien, R. W., Nature *316* (1985) 440.
13 Nilius, B., Hess, P., Lansman, J. B., and Tsien, R. W., Nature *316* (1985) 443.
14 Yoshino, M., Someya, T., Nishio, A., Yazawa, K., Usuki, T., and Yabu, H., Pflügers Arch. *414* (1989) 401.
15 Kameyama, M., Hofmann, F., and Trautwein, W., Pflügers Arch. *405* (1985) 285.
16 Vivaudou, M. B., Clapp, L. H., Walsh, J. V. Jr, and Singer, J. J., FASEB J. *2* (1988) 2497.
17 Fish, R. D., Sperti, G., Colucci, W. S., and Clapham, D. E., Circ. Res. *62* (1988) 1049.
18 Yabu, H., Yoshino, M., Usuki, T., Someya, T., Obara, K., Ozaki, H., and Karaki, H., in: Frontiers in Smooth Muscle Research, pp. 623–626. Eds N. Sperelakis and J. D. Wood. Wiley-Liss, New York 1990.
19 Hidaka, H., Inagaki, M., Kawamoto, S., and Sasaki, Y., Biochemistry *23* (1984) 5036.
20 Nishizuka, Y., Nature *308* (1984) 693.

0014-4754/91/090939-03$1.50 + 0.20/0