ELSEVIER

European Journal of Pharmacology 424 (2001) 173–178

ejp

www.elsevier.com/locate/ejphar

Inhibition of acetylcholine-activated K^+ current by chelerythrine and bisindolylmaleimide I in atrial myocytes from mice

Hana Cho [a,b], Jae Boum Youm [c], Yung E. Earm [a,b], Won-Kyung Ho [a,b,*]

[a] *National Research Laboratory for Cellular Signalling, Seoul National University College of Medicine, 28 Yonkeun-Dong, Chongno-Ku, Seoul 110-799, South Korea*

[b] *Department of Physiology and Biophysics, Seoul National University College of Medicine, 28 Yonkeun-Dong, Chongno-Ku, Seoul 110-799, South Korea*

[c] *Department of Physiology, Cheju National University College of Medicine, Ara 1-1, Cheju, South Korea*

Received 15 March 2001; received in revised form 25 June 2001; accepted 29 June 2001

Abstract

The effects of the protein kinase C inhibitors chelerythrine and bisindolylmaleimide I on acetylcholine-activated K^+ currents (I_{KACh}) were examined in atrial myocytes of mice, using the patch clamp technique. Chelerythrine and bisindolylmaleimide I inhibited I_{KACh} in a reversible and dose-dependent manner. Half-maximal effective concentrations were 0.49 ± 0.01 μM for chelerythrine and 98.69 ± 12.68 nM for bisindolylmaleimide I. However, I_{KACh} was not affected either by calphostin C, which is also known as a protein kinase C inhibitor, or by a protein kinase C activator, phorbol 12,13-dibutyrate. When K_{ACh} channels were activated directly by adding 1 mM GTPγS to the bath solution in inside-out patches, chelerythrine (10 μM) decreased the open probability from 0.043 ± 0.01 to 0.014 ± 0.007 ($n = 5$), but bisindolylmaleimide I did not affect the channel activity. From these results, it is concluded that both chelerythrine and bisindolylmaleimide I inhibit K_{ACh} channels independently of protein kinase C inhibition, but the level of inhibition is different.

Keywords: Chelerythrine; Bisindolylmaleimide I; Protein kinase C; Acetylcholine-activated K^+ channel; Atrial myocyte; Electrophysiology

1. Introduction

Protein kinase C is an enzyme that phosphorylates the hydroxyl group of serine and/or threonine (Ser/Thr) residues in various protein substrates, and this process is considered to be a fundamental regulatory mechanism involved in cellular growth, differentiation and immediate regulation of effector functions (Hug and Sarre, 1993; Nishizuka, 1995; Sugden and Bogoyevitch, 1995; Steinberg et al., 1995). In cardiac myocytes, protein kinase C is suggested to be involved in cardiac hypertrophy (Steinberg et al., 1995) and anoxic preconditioning (Zhu et al., 2000; Ikonomidis et al., 1997; Zhou et al., 1996). Protein kinase C has also been identified as a regulator of intracellular Ca^{2+} and cardiac contractility (Woo and Lee, 1999; Capogrossi et al., 1990). Ion channels can be targets for protein kinase C action, and the activation of cardiac ATP-sensitive K^+ channels by protein kinase C has been reported (Hu et al., 1996; Light et al., 1996).

For investigating protein kinase C-mediated signal transduction, biochemical inhibitors have been widely used in both in vivo and in vitro studies. Bisindolylmaleimide I (GF 109203X) and chelerythrine are known as potent and selective protein kinase C inhibitors. However, their usefulness in intact cells can be limited by non-specific actions on other targets. This limitation is evident in recent reports identifying inhibition by bisindolylmaleimides of K_V 1.5 channels (Choi et al., 2000) and inhibition by chelerythrine of acetylcholine-induced currents in PC12 cells (Shi and Wang, 1999), with potencies greater than or comparable to those for inhibition of protein kinase C (IC_{50} values in the nM range). Considering the significance of protein kinase C in myocardial function, information about the direct action of protein kinase C inhibitors on cardiac ion channels is necessary for the right interpretation of experimental results obtained with these substances. In the present study, we demonstrate that bisindolylmaleimide I and chelerythrine directly inhibit

* Corresponding author. Department of Physiology and Biophysics, Seoul National University College of Medicine, 28 Yonkeun-Dong, Chongno-Ku, Seoul 110-799, South Korea. Tel.: +82-2-740-8227; fax: +82-2-763-9667.

E-mail address: wonkyung@snu.ac.kr (W.-K. Ho).

0014-2999/01/$ - see front matter
PII: S0014-2999(01)01169-4

acetylcholine-activated K^+ (K_{ACh}) channels in atrial myocytes of mice at concentrations similar to those used for protein kinase C inhibition.

2. Materials and methods

2.1. Cell isolation

Mouse atrial myocytes were isolated by perfusing Ca^{2+}-free normal Tyrode solution containing collagenase (0.14 mg ml^{-1}, Sigma Type 5) on a Langendorff column at 37 °C, as previously described (Cho et al., 2001). Isolated atrial myocytes were kept in high-K^+ and low-Cl^- solution at 4 °C until use.

2.2. Solutions

Normal Tyrode solution contained (mM): 143 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 1.8 $CaCl_2$, 10 glucose, 5 HEPES, titrated to pH 7.4 with NaOH. In the presence of acetylcholine, 10 μM glibenclamide was applied to inhibit the ATP-sensitive K^+ channel during acetylcholine application (Shui et al., 1995). The pipette solution for perforated patches contained (mM): 140 KCl, 10 HEPES, 1 $MgCl_2$, 5 EGTA, titrated to pH 7.2 with KOH. The high-K^+ and low-Cl^- solution contained (mM): 50 L-glutamate, 50 KCl, 20 taurine, 20 KH_2PO_4, 3 $MgCl_2$, 20 glucose, 10 HEPES, 0.5 EGTA, titrated to pH 7.4 with KOH.

For single-channel experiments, the bath solution contained (mM): 140 KCl, 5 EGTA, 1 $MgCl_2$, 5 HEPES, 5 glucose, pH 7.4 (with KOH). GTPγS (1 mM) and ATP (3 mM) were added in test bath solution. The pipette solution contained (mM): 140 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 HEPES, pH 7.4 (with KOH).

2.3. Chemicals

Acetylcholine (Sigma) and chelerythrine (RBI) were dissolved in deionized water to make a stock solution (10 mM) and stored at −20 °C. On the day of experiments, one aliquot was thawed and used. Bisindolylmaleimide I (RBI), calphostin C (Biomol), phorbol 12,13-dibutyrate (PDB; Biomol) were first dissolved in dimethyl sulfoxide as a stock solution and then used at the final concentration in the solution. Final concentrations of dimethyl sulfoxide did not exceed 0.1% and were without effect on K_{ACh} currents. All experiments were conducted at 35 ± 1 °C.

2.4. Voltage-clamp technique

Whole-cell currents were recorded from single isolated myocytes in a perforated patch configuration using nystatin (Sigma, 200 μg/ml). Voltage clamp was performed using an Axopatch-1C amplifier (Axon instruments). After the gigaseal was made, we usually waited 10–15 min until the series resistance decreased below 10 MΩ to allow the successful whole-cell configuration. In most cells, this condition remained stable during the whole experimental period. If the series resistance began to increase, we stopped recording and the data were discarded. Data were digitized with pClamp software 5.7.1 (Axon instruments) at a sampling rate of 1–2 kHz. For single-channel experiments, fire-polished pipettes (5–6 MΩ) were used. Channel activity was monitored at −80 mV at a sampling rate of 5 kHz, and filtered at 1 kHz. The results in the text and in the figures are presented as means ± S.E.M. (n = number of cells tested). Statistical analyses were performed using Student's t-test.

3. Results

3.1. Chelerythrine and bisindolylmaleimide I inhibited the whole-cell IKACh

Acetylcholine-activated K^+ currents (I_{KACh}) were recorded from a single isolated atrial myocyte using the nystatin-perforated patch clamp technique (Fig. 1A). When the membrane potential was held at −40 mV, application of 10 μM acetylcholine induced a rapid activation of the

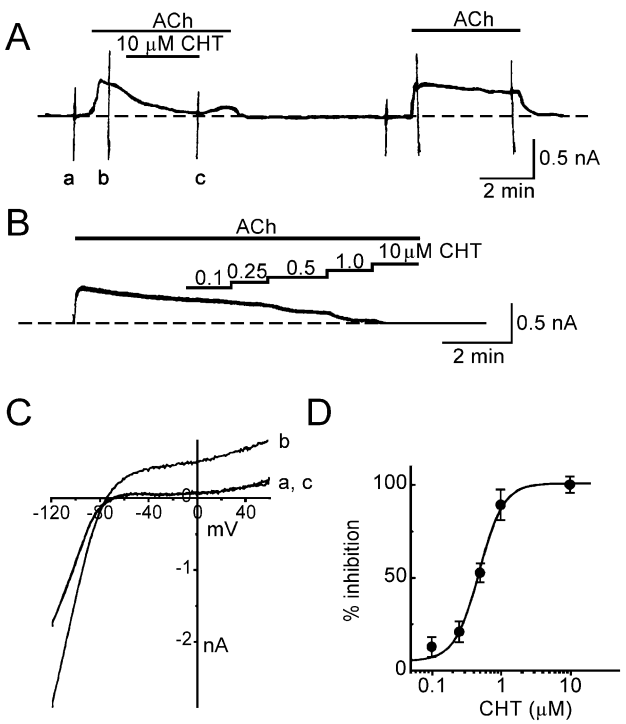

Fig. 1. Chelerythrine (CHT) inhibits I_{KACh}. Chart recordings of the perforated patch whole-cell current at a holding potential of −40 mV. The dotted line indicates zero current level. (A) The additions of 10 μM acetylcholine (ACh) and 10 μM chelerythrine are indicated by the horizontal bar above the trace. The vertical deflections of current trace are the responses to voltage ramps. (B) The additions of 10 μM acetylcholine and chelerythrine are indicated by the horizontal bars above the trace. (C) I–V relationships obtained at the points indicated by a–c in A. (D) Dose–response relationship for chelerythrine. The amplitude of steady-state I_{KACh} in the absence of chelerythrine was regarded as control. The percent inhibition was plotted against chelerythrine concentration.

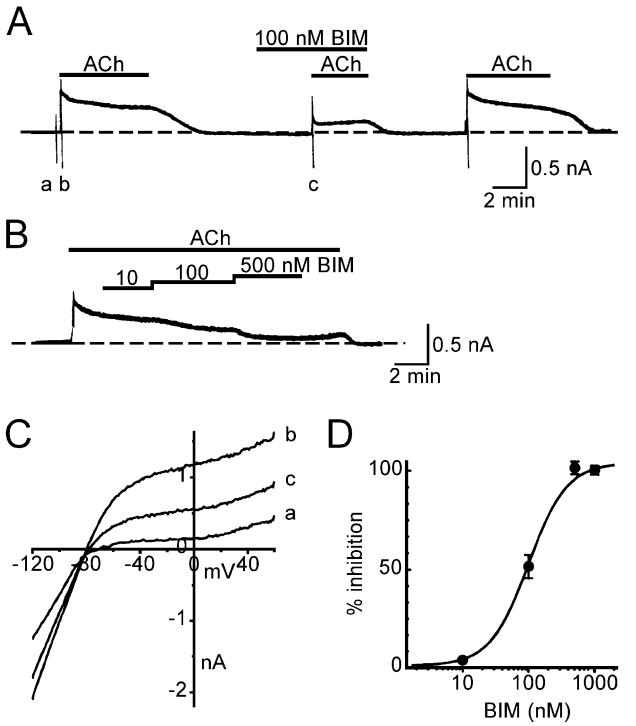

Fig. 2. Bisindolylmaleimide I (BIM) inhibits the acetylcholine-activated K^+ current. Chart recordings of the perforated patch whole-cell current at a holding potential of −40 mV. The dotted line indicates zero current level. (A) The additions of 10 μM acetylcholine (ACh) and 100 nM bisindolylmaleimide I are indicated by the horizontal bars above the trace. The vertical deflections of current trace are the responses to voltage ramps. (B) The additions of 10 μM acetylcholine and bisindolylmaleimide I are indicated by the horizontal bars above the trace. (C) *I–V* relationships obtained at the points indicated by a–c in A. (D) Dose–response relationship for bisindolylmaleimide I. The amplitude of steady-state I_{KACh} in the absence of bisindolylmaleimide I was regarded as control. The percent inhibition was plotted against bisindolylmaleimide I concentration.

outward I_{KACh} followed by a decrease in the current due to desensitization, as was previously reported (Cho et al., 2001). When chelerythrine was added after I_{KACh} reached quasi-steady-state, I_{KACh} decreased markedly (Fig. 1A). The inhibition by chelerythrine was reversible and I_{KACh} had fully recovered by the second application of acetylcholine 6 min after washout.

Current–voltage (*I–V*) curves were obtained from the current response induced by voltage ramps between +60 and −120 mV (at a speed of ±0.6 V s^{-1}) from the holding potential of −40 mV. The ramps were applied before acetylcholine application (a), at steady state of I_{KACh} (b), and I_{KACh} in the presence of chelerythrine (c), as indicated in Fig. 1A. Corresponding *I–V* curves were plotted in Fig. 1C: *I–V* relationships demonstrated that chelerythrine inhibited the net I_{KACh} current over the whole voltage range tested, and that the background inward rectifying K^+ current (IRK) was not significantly affected by chelerythrine.

The dose–response relationship for chelerythrine was examined in Fig. 1B. Various concentrations of chelerythrine were applied in a cumulative manner when I_{KACh} reached a quasi-steady-state level. The amplitude of I_{KACh} was measured at −40 mV before and during the application of chelerythrine. The percent inhibition in the presence of chelerythrine was calculated with respect to the amplitude of steady-state I_{KACh} in the absence of chelerythrine and plotted in Fig. 1D. The data were fitted with the Hill equation, showing that the concentration for half-maximal inhibition (IC_{50}) was 0.49 ± 0.01 μM ($n = 8$). The concentration required for half-maximal inhibition of protein kinase C (IC_{50}) is reported to be 0.66 μM (Herbert et al., 1990).

Other protein kinase C inhibitors were tested. In Fig. 2, bath perfusion of 100 nM bisindolylmaleimide I 3 min before and during a second acetylcholine application significantly attenuated the response to acetylcholine. Bisindolylmaleimide I-mediated inhibition was reversible, as indicated by the response to a third application of 10 μM acetylcholine after washout, which was comparable with the control response. *I–V* relationships were measured before acetylcholine application (a), at peak activation of I_{KACh} (b), and I_{KACh} in the presence of bisindolylmaleimide I (c), as indicated in Fig. 2A. *I–V* relationships demonstrated that bisindolylmaleimide I inhibited the net I_{KACh} current over the whole voltage range tested, and that the background IRK was not significantly affected by

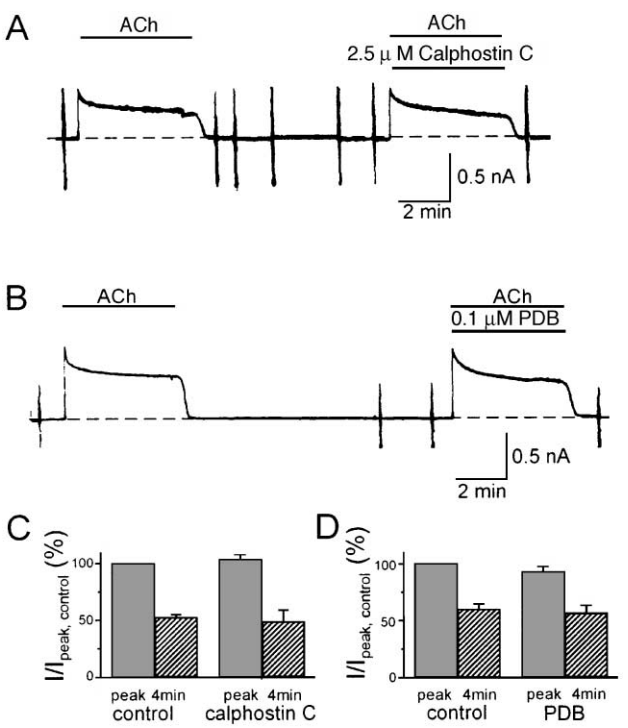

Fig. 3. Effects of 2.5 μM calphostin C (A), 0.1 μM phorbol 12,13-dibutyrate (PDB) (B) on whole-cell K_{ACh} current. Cells were held at −40 mV. The additions of acetylcholine (ACh), calphostin C and phorbol 12,13-dibutyrate are indicated by the bars above the recording. (C) and (D) Summary data of the relative amplitude (percent) of I_{KACh} in respect to the peak I_{KACh} induced by the first application of ACh. Values are means ± S.E.M. for three and four cells, respectively.

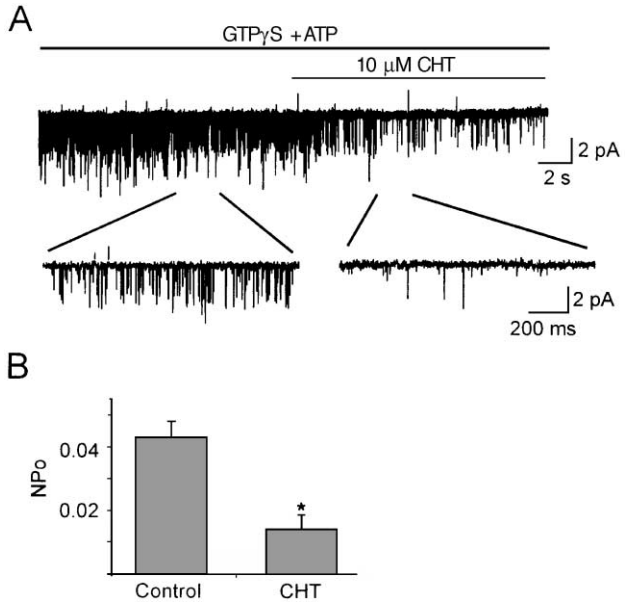

Fig. 4. Effects of 10 μM chelerythrine (CHT) on unitary K_{ACh} current recorded in inside-out membrane patches. (A) K_{ACh} channels in inside-out patches were activated by bath perfusion of 1 mM GTPγS at a holding potential of −80 mV. (B) Changes in open probability (NP_o) after chelerythrine. Values are means ± S.E.M. for four cells. $^*P < 0.01$ vs. control.

bisindolylmaleimide I (Fig. 2C). The dose–response relationships for bisindolylmaleimide I were also tested with the same method mentioned above (Fig. 2B). The data are summarized in Fig. 2D. The IC_{50} value for inhibition of I_{KACh} was 98.69 ± 12.68 nM ($n = 5$). The IC_{50} value for inhibition of protein kinase C is reported to be 20 nM (Toullec et al., 1991). However, in experiments in which protein kinase C is inhibited, micromolar concentrations have been usually used to overcome the effects of high intracellular protein kinase C concentrations, high ATP concentrations and cell membrane permeability barriers.

Calphostin C (2.5 μM), another membrane-permeable protein kinase C inhibitor, however, had no effect on I_{KACh}, even at a supramaximal concentration for inhibiting protein kinase C ($IC_{50} = 0.05$ μM in Kobayashi et al., 1989; Tamaoki, 1991) ($n = 3$, Fig. 3A and C). Furthermore, PDB, which is known as a potent protein kinase C activator, did not affect the I_{KACh} ($n = 4$, Fig. 3B and D). Data comparing control conditions vs. a treatment with calphostin C or PDB showed no significant difference in I_{KACh}. These results suggest that I_{KACh} inhibition by chelerythrine and bisindolylmaleimide I is exerted not via inhibition of protein kinase C.

3.2. Effects of chelerythrine and bisindolylmaleimide I on the single K_{ACh} channel current

In order to investigate the level at which chelerythrine and bisindolylmaleimide I act to inhibit I_{KACh}, we examined whether they inhibited K_{ACh} channels in excised patches. K_{ACh} channels were directly activated by adding 1 mM GTPγS to the bath solution, so that channel activation by-passed muscarinic receptor activation and the coupling between muscarinic receptors and G-proteins. Fig. 4A shows a representative single-channel recording of K_{ACh} channels from an inside-out patch of atrial membrane held at −80 mV. Usually, two or three channels were included in one patch, but the amplitude of a single-channel opening was well resolved. When the *I–V* relationship for single-channel currents was obtained at various potentials, it showed an inward rectification with a mean slope conductance of 42.4 ± 0.7 pS ($n = 4$) in an inward direction (data not shown). Mean open time under control conditions was 0.76 ± 0.04 ms ($n = 5$) at a patch membrane potential of −80 mV. In the absence of GTPγS, no channel activity with the same property described here was recorded ($n = 7$). These are characteristic features of K_{ACh} channels in heart cells (Kurachi et al., 1990). When 10 μM chelerythrine was applied to the bath solution, the K_{ACh} channel activity was markedly decreased, while the single-channel amplitude was not affected (Fig. 4A). When the channel activity was expressed as NP_o, where N is the number of K_{ACh} channels in the patch membrane and P_o is the open probability, NP_o was decreased from 0.043 ± 0.01 to 0.014 ± 0.007 ($n = 5$, $P < 0.01$, Fig. 4B) by 10 μM chelerythrine. This result suggests that the inhibition by chel-

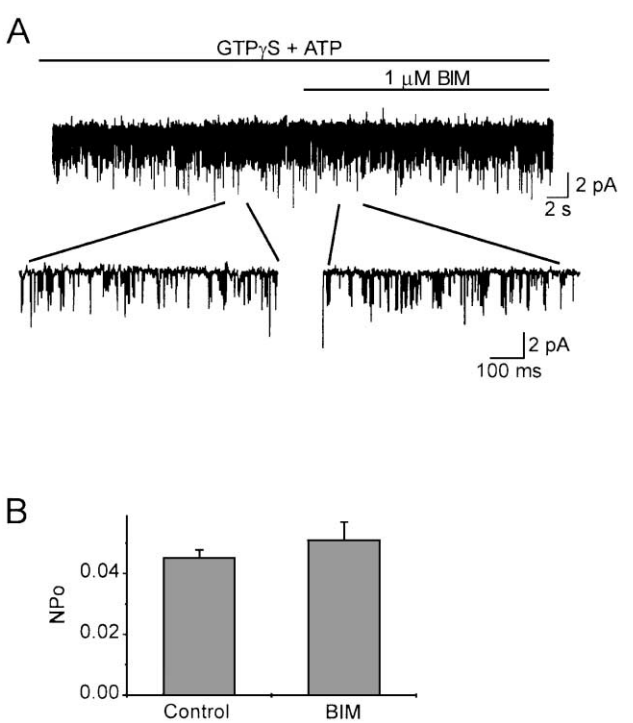

Fig. 5. Effects of 1 μM bisindolylmaleimide I (BIM) on unitary K_{ACh} current recorded in inside-out membrane patches. (A) K_{ACh} channels in inside-out patches were activated by bath perfusion of 1 mM GTPγS at a holding potential of −80 mV. (B) Changes in open probability (NP_o) after bisindolylmaleimide I. Values are means ± S.E.M. for four cells. They were not significantly different ($P > 0.05$).

erythrine of K_{ACh} channels occurs at a level downstream of G-protein activation. Chelerythrine may act either on the coupling between the G-protein and the K_{ACh} channel, or on the K_{ACh} channel itself.

The effect of bisindolylmaleimide I was also tested with the same method mentioned above. As shown in Fig. 5A, 1 μM bisindolylmaleimide I did not affect single-channel activity recorded from excised-out patches. The discrepancy between whole-cell results and single-channel results suggest that the inhibition of I_{KACh} by bisindolylmaleimide I occurs at a level upstream from the G-protein, possibly at the level of the muscarinic receptor. The results obtained from three cells are summarized in Fig. 5B.

4. Discussion

The results of the present study can be summarized as follows: (1) chelerythrine and bisindolylmaleimide I inhibit I_{KACh} in cardiac myocytes at concentrations commonly used in experiments investigating the role of protein kinase C. (2) Other protein kinase C inhibitors and activators, including calphostin C and PDBu, did not inhibit I_{KACh}, thus ruling out the possibility that protein kinase C is involved in the inhibition of K_{ACh} channels by chelerythrine and bisindolylmaleimide I. (3) Inhibition of K_{ACh} channels by chelerythrine also occurred in inside-out patches when channels were activated directly by adding 1 mM GTPγS to the bath solution. (4) Bisindolylmaleimide I, however, did not affect K_{ACh} channels in inside-out patches. (5) The background IRK were not affected by chelerythrine and bisindolylmaleimide I. These results demonstrate that the effects of chelerythrine and bisindolylmaleimide I are not attributable to protein kinase C inhibition. The mechanism of channel inhibition by chelerythrine and bisindolylmaleimide I was not thoroughly investigated in the present study, but the above results imply that chelerythrine acts either on the coupling between G-protein and G-protein-activated channels, or on the ion channel itself. In contrast, bisindolylmaleimide I exerts its effect upstream of the G-protein, at the muscarinic receptor.

Chelerythrine, a benzophenanthridine alkaloid, is a selective protein kinase C inhibitor with an IC_{50} of 0.66 μM. The inhibitory effects of chelerythrine on other kinases such as protein kinase A, Ca^{2+}/calmodulin-dependent protein kinase, and tyrosine protein kinase are considerably less potent, with IC_{50} values ranging from 100 to 170 μM (Herbert et al., 1990). Bisindolylmaleimide I is also well known as a specific protein kinase C inhibitor ($IC_{50} = 20$ nM). However, effects of chelerythrine and bisindolylmaleimide I on targets other than protein kinase C have also been identified recently. Chelerythrine inhibits alanine aminotransferase ($IC_{50} = 4$ μM) (Walterova et al., 1981), Na,K-ATPase ($IC_{50} = 30–50$ μM) (Cohen et al., 1978) and acetylcholine-induced currents in PC 12 cells (Shi and Wang, 1999). Bisindolylmaleimide I inhibits voltage-dependent Na^+ channels (Lingameneni et al., 2000), K_V 1.5 channels (Choi et al., 2000) and 5-HT_3 receptors (Coultrap et al., 1999) with a potency comparable to that for inhibition of protein kinase C, and chromaffin cell nicotinic receptors (Marley and Thomson, 1996) and human muscarinic receptors (Lazareno et al., 1998) at higher concentrations. Inhibition of K_{ACh} channels must be added to the growing list of additional effects of these specific inhibitors.

The pathophysiological roles of the protein kinase C-mediated signal transduction pathway have been demonstrated in cardiac tissue. In these studies, chelerythrine and bisindolylmaleimide I were widely used. Speechly-Dick et al. (1994) reported that in an in vivo ischemia model, the protein kinase C inhibitor chelerythrine, administered after a preconditioning stimulus, abolished the protection conferred by ischemic preconditioning, and caused an increase in infarct size. Eble et al. (2000) demonstrated that pretreatment with chelerythrine completely blocked endothelin-induced focal adenosine kinase phosphorylation, suggesting that protein kinase C is involved in endothelin-induced cardiac hypertrophy. The present study, however, suggests that chelerythrine and bisindolylmaleimide I may not be suitable as pharmacological probes to investigate the role of protein kinase C in the regulation of physiological processes in the heart. Previous studies that used chelerythrine and bisindolylmaleimide I must be re-evaluated in the light of the present results.

In conclusion, chelerythrine and bisindolylmaleimide I, which have often been used in signal transduction pathway research as protein kinase C inhibitors, are also able to inhibit K_{ACh} channels in mouse atrial myocytes.

Acknowledgements

This work was supported by BK21 Human Life Sciences and by R&D project from the Ministry of Science and Technology. We thank Dr. S.H. Lee for helpful discussion and critical reading of the manuscript.

References

Capogrossi, M.C., Kaku, T., Filburn, C.R., Pelto, D.J., Hansford, R.G., Spurgeon, H.A., Lakatta, E.G., 1990. Phorbol ester and dioctanoylglycerol stimulate membrane association of protein kinase C and have a negative inotropic effect mediated by changes in cytosolic Ca^{2+} in adult rat cardiac myocytes. Circ. Res. 66, 1143–1155.

Cho, H., Nam, G.B., Lee, S.H., Earm, Y.E., Ho, W.K., 2001. Phosphatidylinositol 4,5-bisphosphate is acting as a signal molecule in α_1-adrenergic pathway via the modulation of acetylcholine-activated K^+ channels in mouse atrial myocytes. J. Biol. Chem. 276, 159–164.

Choi, B.H., Choi, J.S., Jeong, S.W., Hahn, S.J., Yoon, S.H., Jo, Y.H., Kim, M.S., 2000. Direct block by bisindolylmaleimide of rat Kv1.5 expressed in Chinese hamster ovary cells. J. Pharmacol. Exp. Ther. 293, 634–640.

Cohen, H.G., Seifen, E.E., Straub, K.D., Tiefenback, C., Stermitz, F.R., 1978. Structural specificity of the NaK-ATPase inhibition by sanguinarine, an isoquinoline benzophenanthridien alkaloid. Biochem. Pharmacol. 27, 2555–2558.

Coultrap, S.J., Sun, H., Tenner, T.E., Machu, T.K., 1999. Competitive antagonism of the mouse 5-hydroxytryptamine$_3$ receptor by bisindolylmaleimide I, a "selective" protein kinase C inhibitor. J. Pharmacol. Exp. Ther. 290, 76–82.

Eble, D.M., Strait, J.B., Govindarajan, G., Lou, J., Byron, K.L., Samarel, A.M., 2000. Endothelin-induced cardiac myocyte hypertrophy: role for focal adhesion kinase. Am. J. Physiol. 278, H1695–H1707.

Herbert, J.M., Augereau, J.M., Gleye, J., Maffrand, J.P., 1990. Chelerythrine is a potent and specific inhibitor of protein kinase C. Biochem. Biophys. Res. Commun. 172, 993–999.

Hu, K., Duan, D., Li, G.R., Nattel, S., 1996. Protein kinase C activates ATP-sensitive K^+ current in human and rabbit ventricular myocytes. Circ. Res. 78, 492–498.

Hug, H., Sarre, T.F., 1993. Protein kinase C isoenzymes: divergence in signal transduction? Biochem. J. 291, 329–343.

Ikonomidis, J.S., Shirai, T., Weisel, R.D., Derylo, B., Rao, V., Whiteside, C.I., Mickle, D.A., Li, R.K., 1997. Preconditioning cultured human pediatric myocytes requires adenosine and protein kinase C. Am. J. Physiol. 272, H1220–H1230.

Kobayashi, E., Nakano, H., Morimoto, M., Tamaoki, T., 1989. Calphostin C (UCN-1028C), a novel microbial compound, is highly potent and specific inhibitor of protein kinase C. Biochem. Biophys. Res. Commun. 159, 548–553.

Kurachi, Y., Ito, H., Sugimoto, T., 1990. Positive cooperativity in activation of the cardiac muscarinic K^+ channel by intracellular GTP. Pfluegers Arch. 416, 216–218.

Lazareno, S., Popham, A., Birdsall, N.J., 1998. Muscarinic interactions of bisindolylmaleimide analogues. Eur. J. Pharmacol. 360, 281–284.

Light, P.E., Sabir, A.A., Allen, B.G., Walsh, M.P., French, R.J., 1996. Protein kinase C-induced changes in the stoichiometry of ATP binding activate cardiac ATP-sensitive K^+ channels. A possible mechanistic link to ischemic preconditioning. Circ. Res. 79, 399–406.

Lingameneni, R., Vysotskaya, T.N., Duch, D.S., Hemmings, H.C., 2000. Inhibition of voltage-dependent sodium channels by Ro 31-8220, a 'specific' protein kinase C inhibitor. FEBS Lett. 473, 265–268.

Marley, P.D., Thomson, K.A., 1996. Inhibition of nicotinic responses of bovine adrenal chromaffin cells by the protein kinase C inhibitor, Ro 31-8220. Br. J. Pharmacol. 119, 416–422.

Nishizuka, Y., 1995. Protein kinase C and lipid signaling for sustained cellular responses. FASEB J. 9, 484–496.

Shi, L., Wang, Ca., 1999. Inhibitory effect of the kinase inhibitor chelerythrine on acetylcholine-induced current in PC12 cells. Arch. Biochem. Biophys. 368, 40–44.

Shui, Z., Boyett, M.R., Zang, W.J., Haga, T., Kameyama, K., 1995. Receptor kinase-dependent desensitization of the muscarinic K^+ current in rat atrial cells. J. Physiol. 487, 359–366.

Speechly-Dick, M.E., Mocanu, M.M., Yellon, D.M., 1994. Protein kinase C. Its role in ischemic preconditioning in the rat. Circ. Res. 75, 586–590.

Steinberg, S.F., Goldberg, M., Rybin, V.O., 1995. Protein kinase C isoform diversity in the heart. J. Mol. Cell. Cardiol. 27, 141–153.

Sugden, P.H., Bogoyevitch, M.A., 1995. Intracellular signalling through protein kinases in the heart. Cardiovasc. Res. 30, 478–492.

Tamaoki, T., 1991. Use and specificity of staurosprine UCN-01, and calphostin C as protein kinase inhibitors. Methods Enzymol. 201, 340–347.

Toullec, D., Pianetti, P., Coste, H., Bellevergue, P., Grand-Perret, T., Ajakane, M., Baudet, V., Boissin, P., Boursier, E., Loriolle, F., 1991. The bisindolylmaleimide GF 109203X is a potent and selective inhibitor of protein kinase C. J. Biol. Chem. 266, 15771–15781.

Walterova, D., Ulrichova, J., Preininger, V., Simanek, V., Lenfeld, J., Lasovsky, J., 1981. Inhibition of liver alanine aminotransferase activity by some benzophenanthridine alkaloids. J. Med. Chem. 24, 1100–1103.

Woo, S.H., Lee, C.O., 1999. Role of PKC in the effects of alpha1-adrenergic stimulation on Ca^{2+} transients, contraction and Ca^{2+} current in guinea-pig ventricular myocytes. Pfluegers Arch. 437, 335–344.

Zhou, X., Zhai, X., Ashraf, M., 1996. Preconditioning of bovine endothelial cells. The protective effect is mediated by an adenosine A2 receptor through a protein kinase C signaling pathway. Circ. Res. 78, 73–81.

Zhu, Z., Li, Y.L., Li, D.P., He, R.R., 2000. Effect of anoxic preconditioning on ATP-sensitive potassium channels in guinea-pig ventricular myocytes. Pfluegers Arch. 439, 808–813.