British Journal of Pharmacology (1998) 124, 600–606
http://www.stockton-press.co.uk/bjp

Effects of κ-opioid receptor stimulation in the heart and the involvement of protein kinase C

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1 The role of protein kinase C (PKC) in mediating the action of κ-receptor stimulation on intracellular Ca^{2+} and cyclic AMP production was determined by studying the effects of *trans*-(±)-3,4-dichloro-N-methyl-N-(2-[1-pyrrolidinyl] cyclohexyl) benzeneacetamide methanesulphonate (U50,488H), a selective κ-receptor agonist, and phorbol 12-myristate 13-acetate (PMA), a PKC agonist, on the electrically-induced $[Ca^{2+}]_i$ transient and forskolin-stimulated cyclic AMP accumulation in the presence and absence of a PKC antagonist, staurosporine or chelerythrine, in the single rat ventricular myocyte.

2 U50,488H at 2.5–40 μM decreased both the electrically-induced $[Ca^{2+}]_i$ transient and forskolin-stimulated cyclic AMP accumulation dose-dependently, effects which PMA mimicked. The effects of the κ-agonist, that were blocked by a selective κ-antagonist, nor-binaltorphimine, were significantly antagonized by the PKC antagonists, staurosporine and/or chelerythrine. The results indicate that PKC mediates the actions of κ-receptor stimulation.

3 To determine whether the action of PKC was at the sarcoplasmic reticulum (SR) or not, the $[Ca^{2+}]_i$ transient induced by caffeine, that depletes the SR of Ca^{2+}, was used as an indicator of Ca^{2+} content in the SR. The caffeine-induced $[Ca^{2+}]_i$ transient was significantly reduced by U50,488H at 20 μM. This effect of U50,488H on caffeine-induced $[Ca^{2+}]_i$ transient was significantly attenuated by 1 μM chelerythrine, indicating that the action of PKC involves mobilization of Ca^{2+} from the SR. When the increase in IP_3 production in response to κ-receptor stimulation with U50,488H in the ventricular myocyte was determined, the effect of U50,488H was the same in the presence and absence of staurosporine, suggesting that the effect of PKC activation subsequent to κ-receptor stimulation does not involve IP_3. The observations suggest that PKC may act directly at the SR.

4 In conclusion, the present study has provided evidence for the first time that PKC may be involved in the action of κ-receptor stimulation on Ca^{2+} in the SR and cyclic AMP production, both of which play an essential role in Ca^{2+} homeostasis in the heart.

Keywords: U50,488H; κ-opioid receptor; protein kinase C; cyclic AMP; intracellular Ca^{2+}; ventricular myocyte; rat isolated heart

Introduction

κ-Opioid receptors are present in the myocardium (Krumins *et al*., 1985; Tai *et al*., 1991; Zhang *et al*., 1996) and κ-receptor stimulation decreases cardiac contraction (Xia *et al*., 1994). Recently, it has been shown that κ-receptor stimulation with a selective agonist, *trans*-(±)-3,4-dichloro-N-methyl-N-(2-[1-pyrrolidinyl] cyclohexyl) benzeneacetamide methanesulphonate (U50,488H), reduces the electrically-induced $[Ca^{2+}]_i$ transient, which has been shown to correlate directly with the contractility of the heart (Tatsukawa *et al*., 1993; Janczewski & Lakatta, 1993). The reduction in the electrically-induced $[Ca^{2+}]_i$ transient results from mobilization of Ca^{2+} from sarcoplasmic reticulum (SR) (Ventura *et al*., 1992), which reduces the availability of Ca^{2+} for release from the intracellular store in response to electrical stimulation. The mobilization of Ca^{2+} from its intracellular store is secondary to an increased production of inositol 1,4,5-trisphosphate (IP_3), a breakdown product of phosphatidylinositol 4,5-biphosphate (PIP_2) under the action of phospholipase C (PLC), upon κ-receptor stimulation (Ventura *et al*., 1992; Sheng *et al*., 1996). κ-Receptor stimulation also reduces adenosine 3′,5′-cyclic monophosphate (cyclic AMP) production (Zhang & Wong, 1998), which is believed to be responsible for the reduced cardiac contractility (Hohl & Li, 1991). So activation of the PLC-IP_3/Ca^{2+} pathway and suppression of the adenylate cyclase (AC)/cyclic AMP pathway upon κ-receptor stimulation may be responsible, at least partly, for the reduced contractility.

Protein kinase C (PKC), which is activated by diacylglycerol (DAG; Nishizuka, 1992), another breakdown product of PIP_2 under the action of PLC, has been shown to be involved in Ca^{2+} regulation by several receptors in the heart (Kem *et al*., 1991; Capogrossi *et al*., 1991). A previous study by Ventura and co-workers (1991) showed that in the heart U50,488H activates PKC and increases cytosolic pH and the effects were blocked by a PKC antagonist, staurosporine. The observations suggest that PKC also mediates the action of κ-receptor stimulation in the heart. However, the role of PKC in Ca^{2+} regulation upon κ-receptor stimulation is not known. To address this question, we studied the cardiac effects of κ-opioid receptor stimulation with U50,488H and activation of PKC with phorbol 12 -myristate 13-acetate (PMA), a PKC agonist, in the presence and absence of a PKC antagonist, staurosporine or chelerythrine. We measured the forskolin-stimulated cyclic AMP accumulation, IP_3 production and the Ca^{2+} transients induced by electrical stimulation and caffeine. Electrical stimulation mimics the arrival of an action potential generated from the sino-atrial node of the heart, both triggering the same cascade of events-membrane depolarization, influx of Ca^{2+} and Ca^{2+} release from the SR-leading to

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muscle contraction (Alexandre, 1983). Caffeine activates the Ca^{2+} release channel of the SR (Sitsapesan & Williams, 1990; Bassani *et al*., 1993) and therefore the caffeine-induced Ca^{2+} transient can be used as an index of Ca^{2+} content in the SR (Ventura *et al*., 1992; Wang *et al*., 1997). Results from the present study suggest that PKC may mediate the actions of κ-receptor stimulation on intracellular Ca^{2+} by acting at the level of SR and by reducing the production of cyclic AMP.

Methods

Measurement of $[Ca^{2+}]_i$

Ventricular myocytes were isolated from the heart of male Sprague-Dawley rats (190–210 g), by a collagenase perfusion method described previously (Dong *et al*., 1993). Immediately after decapitation, the hearts were rapidly removed from the rat and perfused in a retrograde manner at a constant flow rate (10 ml min^{-1}) with oxygenated Joklik modified Eagle's medium supplemented with 1.25 mM $CaCl_2$ and 10 mM HEPES, pH 7.2, at 37°C for 5 min followed by 5 min with the same medium free of Ca^{2+}. Collagenase was then added to the medium to a concentration of 125 u ml^{-1} with 0.1% (w/v) bovine serum albumin (BSA). After 35–45 min of perfusion with medium containing collagenase, the atria were discarded and the ventricular tissue was dissociated by shaking in the same oxygenated collagenase solution for 5 min at 37°C. The ventricular tissue was cut into small pieces with a pair of scissors followed by stirring with a glass rod for 5 min, which separated the ventricular myocytes from each other. The residue was filtered through 250 μm mesh screens, sedimented by centrifugation at 100 *g* for 1 min and resuspended in fresh Joklik solution with 2% BSA. More than 70% of the cells were rod-shaped and impermeable to trypan-blue. Ca^{2+} concentration of the Joklik solution was increased gradually to 1.25 mM in 30 min.

Ventricular myocytes were incubated with fura-2/AM (4 μM) in Joklik solution supplemented with 1.25 mM $CaCl_2$ for 25 min. The unincorporated dye was removed by washing the cells twice in fresh incubation solution. The loaded cells were kept at room temperature (24°C–26°C) for 30 min before measurements of $[Ca^{2+}]_i$ to allow the fura-2/AM in the cytosol to de-esterify. Then, the extracellular Ca^{2+} of loaded myocytes were removed by washing with Joklik solution.

The ventricular myocytes loaded with fura-2/AM were transferred to the stage of an inverted microscope (Nikon) in a superfusion chamber at room temperature. The inverted microscope was coupled with a dual-wavelength excitation spectrofluorometer (Photo Technical International, NJ, U.S.A.). The myocytes were perfused with a Krebs bicarbonate buffer (KB buffer) containing (mM): NaCl 117, KCl 5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 1.25, $NaHCO_3$ 25 and glucose 11, with 1% dialyzed BSA and a gas phase of 95% O_2/5% CO_2. The myocytes selected for the study were rod shaped with clear striations. They exhibited a synchronous contraction (twitch) in response to suprathreshold 4 ms stimuli at 0.2 Hz delivered by a stimulator (Grass S88) through two platinum field-stimulation electrodes in the bathing fluid. Fluorescent signals obtained at 340 nm (F340) and at 380 nm (F380) excitation wavelengths were stored in a computer for data processing and analysis. The F340/F380 ratio was used to represent $[Ca^{2+}]_i$ changes in the myocytes.

Assay of cyclic AMP

Samples containing $3 \times 10^6 - 6 \times 10^6$ freshly isolated ventricular myocytes after Ca^{2+} loading were incubated in an atmosphere of 5% CO_2–95% air for 2 h. Five minutes after addition of nor-binaltorphimine (nor-BNI), U50,488H was added and incubated for 10 min followed by addition of forskolin for another 5 min. At the end of treatment, the cells were centrifuged for 5 s at 100 *g*. The medium was aspirated, the sediment was resuspended in ice-cold Krebs solution, and centrifuged again for 5 s at 100 *g*. The supernatant was aspirated. Ice-cold ethanol-HCl (1 ml) was added, mixed and left to stand for 5 min at room temperature. The supernatant was centrifuged at 3000 *g* for 5 min and collected with a pipette. The precipitate was washed with 1 ml of ethanol-water (2 : 1), mixed and centrifuged at 3000 *g* for 5 min. The supernatant was evaporated to dryness at 55°C under a stream of nitrogen. The sediment was stored at −20°C for assay of cyclic AMP. The pellets were neutralized in 0.1 N NaOH for protein determination by the method of Lowry *et al*. (1951), with BSA as a standard.

For determination of cyclic AMP, a competitive binding assay with a kit from Amersham was used. Briefly, 50 μl of 0.5 M Tris (hydroxymethyl) aminomethane (4 mM EDTA) added to 50 μl of each sample on ice, followed by 50 μl of [^{3}H]-cyclic AMP and 100 μl of binding protein. The samples were vortexed for 5 s, placed in an ice bath and allowed to incubate for 2 h. Charcoal suspension (100 μl) was added, and the samples were vortexed for 10 s and centrifuged at 12000 *g* for 2 min at 4°C. Samples of 200 μl of supernatant were removed for scintillation counting.

Assay of IP_3

Ventricular myocytes were resuspended at a final protein concentration of 1.8–2.0 mg ml^{-1} before incubation with the κ-opioid receptor agonist U50,488H (50 μM) for 30 s; previous studies in our laboratory showed that the IP_3 level reached a peak at 30 s (Sheng *et al*., 1996; Sheng & Wong, 1996). At the end of the incubation period, 70 μl of cold 30% $HC1O_4$ was added. After centrifugation at 2500 *g* for 10 min, the pellet was dissolved in 0.3 M NaOH, and the protein content was assayed with BSA as a standard (Lowry *et al*., 1951). The acid supernatant was neutralized with a solution of 1.5 M KOH and 60 mM HEPES adjusted to pH 7.0, and then the IP_3 content was estimated with an IP_3 binding protein ^{3}H assay system (Amersham).

Drugs and chemicals

U50,488H, PMA, staurosporine, chelerythrine chloride, fura-2/AM, type I collagenase and forskolin were purchased from Sigma Chemicals Co. (U.S.A.). Nor-BNI was purchased from Tocris Cookson Ltd. (UK). The dose range of U50,488H used in the present study was 2.5–40 μM as the agonist at this dose range increased $[Ca^{2+}]_i$ and IP_3, as well as suppressing cyclic AMP, and the effects were antagonized by nor-BNI at the range of 1–5 μM (Sheng *et al*., 1997; Zhang *et al*., 1997; Zhang & Wong, 1998), which itself had no effect on any of the preparation studied. The concentrations of the PKC agonist and antagonists used were based on previous studies [PMA (Capogrossi *et al*., 1990; Schluter, 1995), staurosporine (Ventura *et al*., 1991) and chelerythrine (Kandasamy *et al*., 1995)].

[^{3}H]-cyclic AMP and IP_3 assay systems were from Amersham International (U.K.). PMA, staurosporine, cheler-

ythrine chloride, Fura-2/AM and forskolin were dissolved in dimethyl sulphoxide (DMSO) and the rest were dissolved in distilled water. The final concentration of DMSO was 0.1% and at this concentration DMSO had no effect on either $[Ca^{2+}]_i$, cyclic AMP or IP_3.

Statistical analysis

Paired Student's t test was used to determine the difference between control and drug treatment groups. One-way analysis of variance (ANOVA) was used to determine the difference between groups. Significance level was set at $P<0.05$.

Results

Effects of U50,488H and PMA on the electrically-induced $[Ca^{2+}]_i$ transient in the presence and absence of a PKC antagonist, staurosporine

This series of experiments was to determine if PKC mediated the action of κ-receptor stimulation by comparing the effects of κ-receptor stimulation and activation of PKC on the electrically-induced $[Ca^{2+}]_i$ transient in the presence and absence of a PKC antagonist. In agreement with the previous observations (Ventura *et al*., 1992; Sheng *et al*., 1996; 1997),

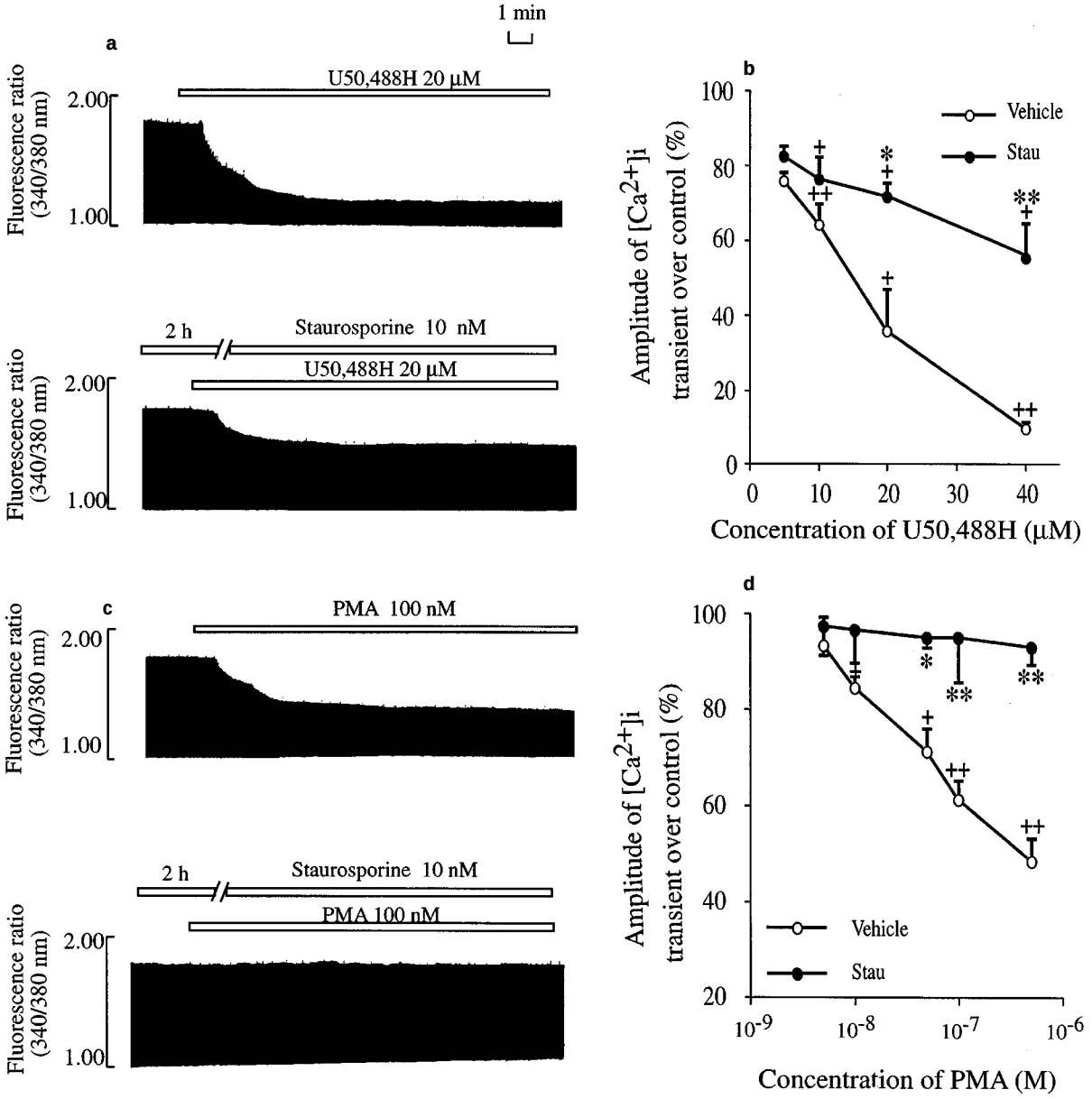

Figure 1 Effects of U50,488H and PMA on the electrically-induced $[Ca^{2+}]_i$ transient in the presence and absence of staurosporine in a single rat ventricular myocyte. (a) Representative tracing showing the effects of 20 μM U50,488H on the amplitude of the $[Ca^{2+}]_i$ transient induced by electrical stimulation at 0.2 Hz in the absence (upper panel) and presence (lower panel) of 10 nM staurosporine. (b) Concentration-response curve of the effects of U50,488H on the electrically-induced $[Ca^{2+}]_i$ transient in a single rat ventricular myocyte pretreated with staurosporine (Stau) for 2 h. (c) Representative tracing showing the effects of a PKC agonist, PMA at 100 nM on the amplitude of $[Ca^{2+}]_i$ transient induced by electrical stimulation at 0.2 Hz in the absence (upper panel) and presence (lower panel) of 10 nM staurosporine. (d) Concentration-response curve of the effect of PMA on the electrically-induced $[Ca^{2+}]_i$ transient in a single rat ventricular myocyte pretreated with staurosporine (Stau) for 2 h. Myocytes were exposed to only one concentration of U50,488H or PMA, and each point represents the mean of 6–8 cells; vertical lines show s.e.mean. All measurements were obtained at 15 min of perfusion with U50,488H or PMA when steady-state conditions were reached. The amplitude of the corresponding $[Ca^{2+}]_i$ transient before drug treatment is 100%. $*P<0.05$, $**P<0.01$ compared with the group without staurosporine. $+P<0.05$, $++P<0.01$ compared with the value before drug treatment.

U50,488H at 5–40 μM dose-dependently reduced the amplitude of the electrically-induced $[Ca^{2+}]_i$ transient in a single ventricular myocyte (Figure 1a, upper panel, and b) and the effect was blocked by 5 μM nor-BNI (data not shown). Interestingly pretreatment of the cells with 10 nM staurosporine for 2 h, which itself had no effect, attenuated significantly the effects of U50,488H (Figure 1a, lower panel, and b).

The upper panel of Figure 1c shows a typical response of the electrically-induced $[Ca^{2+}]_i$ transient to 100 nM PMA in a single ventricular myocyte. In agreement with previous observations (Capogrossi *et al*., 1990; 1991; Kem *et al*., 1991), PMA at 5–500 nM also decreased the amplitude of the electrically-induced $[Ca^{2+}]_i$ transient dose-dependently (Figure 1d) and the effect was abolished completely by pretreatment with 10 nM staurosporine (Figure 1c, lower panel, and d). These responses were similar to those to U50,488H.

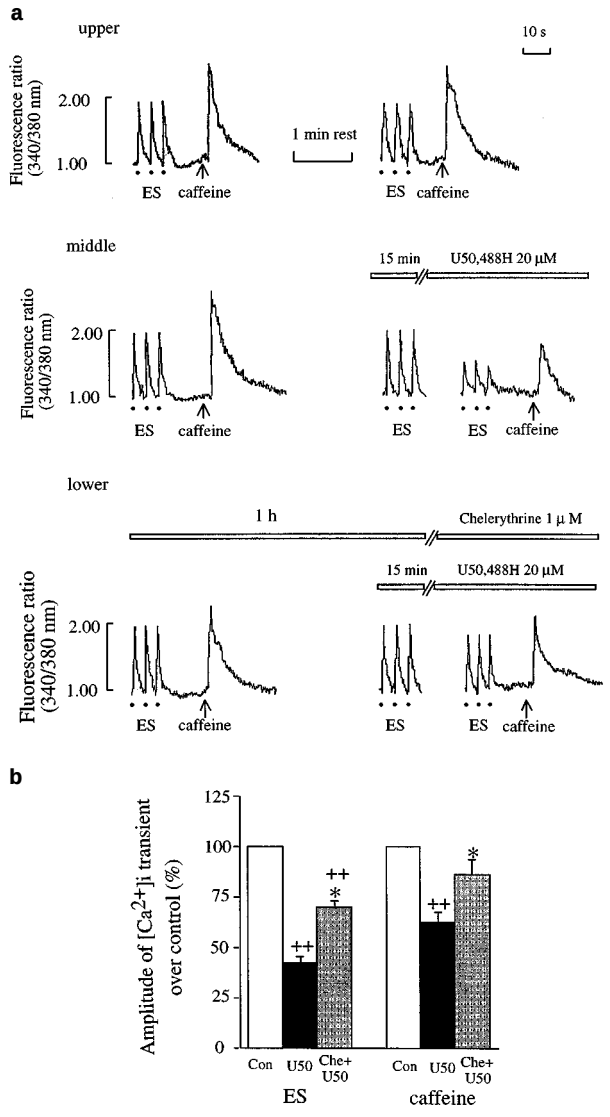

Figure 2 Effects of U50,488H on the caffeine-induced $[Ca^{2+}]_i$ transient in a single rat ventricular myocyte in the presence and absence of chelerythrine. (a) Representative tracings. Upper panel: $[Ca^{2+}]_i$ transients induced by both electrical stimulation (ES) and caffeine. Note that it took 1 min before caffeine was able to induce another $[Ca^{2+}]_i$ transient. Middle panel: $[Ca^{2+}]_i$ transient induced by both electrical stimulation and caffeine in the presence of U50,488H at 20 μM. Lower panel: $[Ca^{2+}]_i$ transient induced by both electrical stimulation and caffeine in the presence of U50,488H at 20 μM following pretreatment with chelerythrine at 1 μM for 1 h. (b) Effects of U50,488H (U50) on electrically (ES) and caffeine-induced $[Ca^{2+}]_i$ transients in a single rat ventricular myocyte in the presence and absence of chelerythrine (Che). The amplitude of the $[Ca^{2+}]_i$ transient before drug treatment is 100%; values are mean ± s.e.mean. $n = 10–11$. $^*P < 0.05$ compared with the group without chelerythrine. $^{++}P < 0.01$ compared with the value before drug treatment.

Effects of U50,488H on the $[Ca^{2+}]_i$ transient induced by caffeine in the presence and absence of a specific PKC antagonist, chelerythrine

The purpose of this experiment was to determine if PKC mediated the action of κ-receptor stimulation on mobilization of Ca^{2+} from the SR, by studying the action of a PKC antagonist on the $[Ca^{2+}]_i$ transient induced by 10 mM caffeine, a Ca^{2+} release channel activator of SR. Like staurosporine, 1 μM chelerythrine, also attenuated the inhibitory effect of U50,488H on the amplitude of the electrically-induced $[Ca^{2+}]_i$ transient (Figure 2). In agreement with previous observations (Ventura *et al*., 1992; Sheng & Wong, 1996), U50,488H attenuated the amplitude of $[Ca^{2+}]_i$ transient induced by 10 mM caffeine (Figure 2a middle panel, and b). Similar to its action on U50,488H-induced inhibition of the electrically-induced $[Ca^{2+}]_i$ transient, chelerythrine also attenuated significantly the inhibitory effects of U50,488H on the amplitude of caffeine-induced $[Ca^{2+}]_i$ transient (Figure 2a lower panel, and b).

Effects of U50,488H on IP_3 production in the presence and absence of staurosporine

In order to determine whether IP_3 is involved in mediating the actions of κ-receptor stimulation via PKC, the effects of 50 μM U50,488H on IP_3 production in the presence and absence of staurosporine in the ventricular myocyte were studied. U50,488H increased the IP_3 level to the same extent in the presence and absence of staurosporine (Figure 3).

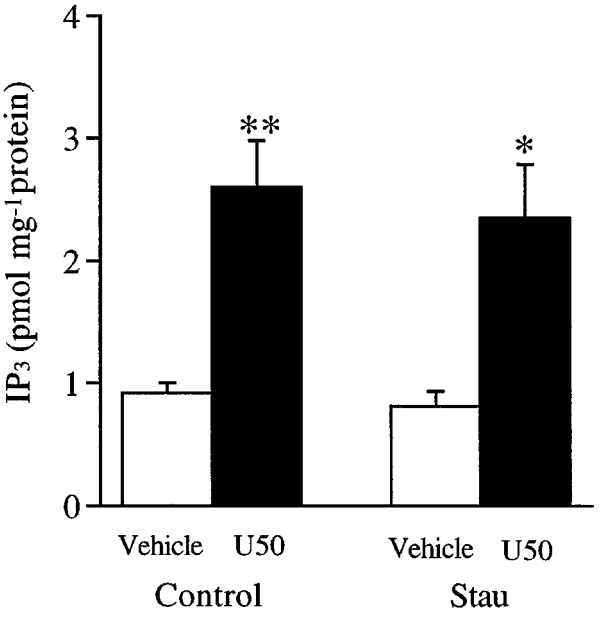

Figure 3 Effects of U50,488H (U50) on IP_3 production in the presence and absence of 10 nM staurosporine (Stau). The PKC antagonist was given 2 h before administration of U50,488H. Thirty seconds after addition of 50 μM U50,488H, cells were fixed for determination of IP_3. Values are mean ± s.e.mean; $n = 6–10$. $^*P < 0.05$, $^{**}P < 0.01$ compared with the control group without U50,488H.

Effects of U50,488H and PMA on the forskolin-induced cyclic AMP accumulation in the presence and absence of staurosporine

In order to determine if PKC also mediated the action of κ-receptor stimulation on cyclic AMP production, the effects of U50,488H and PMA on the forskolin-induced accumulation of cyclic AMP in the presence and absence of staurosporine were studied. In agreement with the well established observations of Zhang and Wong (1998), 10 μM forskolin, an activator of AC, increased cyclic AMP level significantly after 5 min of incubation (Figure 4c). Pretreatment with U50,488H at 2.5–40 μM and PMA at 10 nM – 10 μM dose-dependently decreased the forskolin-stimulated cyclic AMP accumulation (Figure 4a, b). The effects of PMA were completely abolished and those of U50,488H significantly attenuated by 10 nM staurosporine, which itself had no effect on cyclic AMP (Figure 4c). On the other hand, the effects of PMA were not altered by 5 μM nor-BNI, which completely abolished the effect of U50,488H (Figure 4c).

Discussion

The most important observations in the present study are (i), PMA, a PKC agonist mimicked κ-opioid receptor stimulation by decreasing the electrically-induced $[Ca^{2+}]_i$ transient and cyclic AMP production, and (ii) the PKC antagonists, staurosporine and/or chelerythrine, attenuated the effect of κ-receptor stimulation. The observations suggest that the effect of κ-receptor stimulation is mediated at least partly via PKC. This extends the previous observations by Ventura and co-workers (1991) that opioid receptor stimulation activates PKC by providing further evidence that activation of PKC is involved in the regulation of $[Ca^{2+}]_i$ by κ-receptors in the heart.

In order to determine whether PKC mediated the action of κ-receptor stimulation by altering the Ca^{2+} content in the SR, we made use of caffeine, an activator of the Ca^{2+} release channel of the SR, which induces a $[Ca^{2+}]_i$ transient in the ventricular myocyte (Sitsapesan & Williams 1990; Bassani *et al*., 1993). U50,488H attenuated the caffeine-induced $[Ca^{2+}]_i$ transients. The effects of U50,488H on caffeine-induced $[Ca^{2+}]_i$ transients were significantly attenuated by chelerythrine, a specific PKC antagonist. This finding suggests that the action of PKC may involve mobilization of Ca^{2+} from the SR. We next explored the possibility of PKC mediating the action of κ-receptor stimulation via IP_3, which is known to mobilize Ca^{2+} from the SR, by measuring IP_3 production in response to κ-receptor stimulation. We found that the elevation in IP_3 in response to U50,488H was not changed in the presence of staurosporine at a concentration that inhibits the effect of κ-receptor stimulation on the $[Ca^{2+}]_i$ transient, indicating that the effect of PKC activation by κ-receptor stimulation does not involve IP_3 in the present experimental conditions. Therefore, it is likely that PKC acts directly at the SR. This is supported by the finding that activation of PKC decreases the uptake of Ca^{2+} back to the SR in the heart (Rogers *et al*., 1990; Gwathmey & Haijjar, 1990). However, we could not rule out the possibility that PKC increases the responsiveness of the SR to IP_3. Since it takes several minutes for DAG/PKC stimulation to develop fully (Nishizuka, 1992), the present experiment cannot rule out the possibility that PKC would affect IP_3 production when DAG/PKC is fully developed. Further study is needed to address this point.

Since it is well established that cyclic AMP is involved with Ca^{2+} influx in the heart (Sperelakis, 1994), the reduction of cyclic AMP may also contribute to the reduction in the electrically-induced $[Ca^{2+}]_i$ transient. We determined if the suppressive action of κ-receptor stimulation on the production of cyclic AMP also involved PKC. Similar to the effect on $[Ca^{2+}]_i$, the suppressive action of U50,488H on forskolin-induced increase in cyclic AMP was attenuated by staurosporine, indicating that PKC also mediates the action of κ-receptor stimulation on cyclic AMP production. This is in agreement with the finding that activation of PKC decreased cyclic AMP production in cardiomyocytes (Zheng *et al*., 1992; Schluter *et al*., 1995). In a recent study (Zhang & Wong, 1998), we found that the suppressive action of κ-receptor stimulation on cyclic AMP production is due, at least in part, to elevation

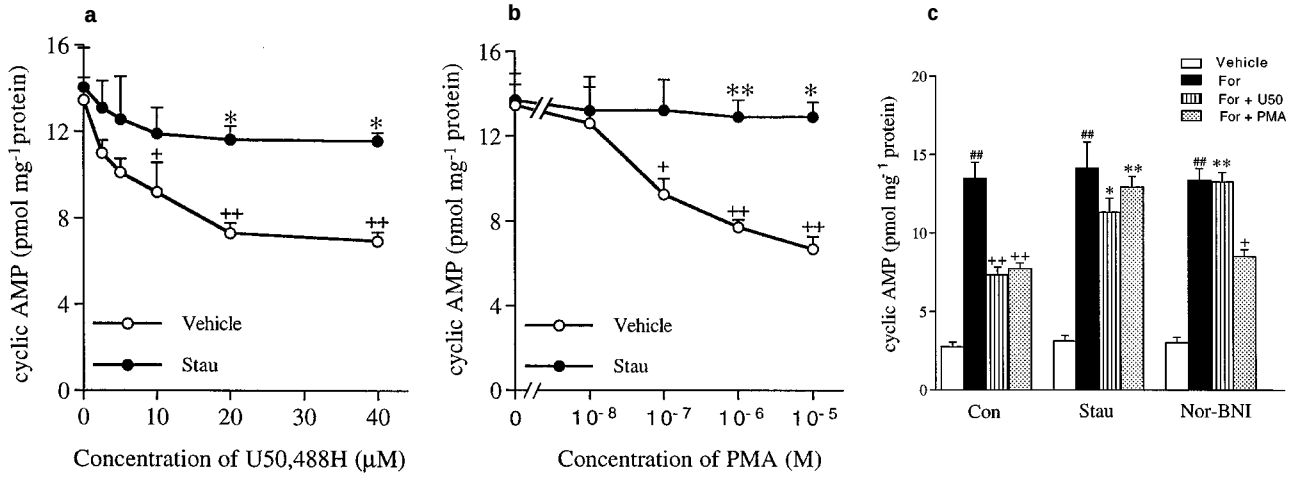

Figure 4 Effects of U50,488H and PMA on forskolin-induced cyclic AMP accumulation in the presence and absence of 10 nM staurosporine. The PKC antagonist was given 2 h before administration of U50,488H. Fifteen minutes after addition of U50,488H, cells were fixed for determination of cyclic AMP. Values are mean of $n=6-15$; vertical lines show s.e.mean. (a) Dose-related response to U50,488H; (b) dose-related response to PMA. $*P<0.05$, $**P<0.01$ compared with the group without staurosporine (Stau). $+P<0.05$, $++P<0.01$ compared with the value before drug treatment. (c) Effects of 20 μM U50,488H (U50) and 1 μM PMA in the presence and absence of staurosporine (Stau) and 5 μM nor-BNI. $*P<0.05$, $**P<0.01$ compared with the corresponding value in the control group. $+P<0.05$, $++P<0.01$ compared with the value of the corresponding forskolin (For)-treated group. $\#P<0.05$, $\#\#P<0.01$ compared with the value of the corresponding vehicle-treated group.

of $[Ca^{2+}]_i$ resulting from the activation of the phosphoinositol/Ca^{2+} pathway. The present study showed that in addition to the Ca^{2+} release from the SR, PKC also mediates the suppressive action of κ-receptor stimulation of cyclic AMP production. This finding also explains the previous observation that the inhibitory effect of a simple elevation of $[Ca^{2+}]_i$ by the action of A-23187, a Ca^{2+} ionophore, on cyclic AMP is much less potent than that of κ-receptor stimulation (Zhang & Wong, 1998).

Previous studies have shown that cardiac κ-receptor stimulation results in a reduction in contractility of the heart and arrhythmias, both of which are believed to be due to alterations in Ca^{2+} homeostasis in the heart (Ventura *et al*., 1992; Wong *et al*., 1995). The alterations in Ca^{2+} homeostasis have been shown to result from suppression of the AC/cyclic AMP pathway and activation of the PLC-IP_3/Ca^{2+} pathway (Ventura *et al*., 1992; Sheng *et al*., 1997; Zhang & Wong, 1998). The present study provides evidence for the first time that PKC is also involved in mediating the actions of cardiac κ-receptor stimulation on Ca^{2+} homeostasis. The site of action may be at the level of SR and formation/degradation of cyclic AMP. In order to confirm that PKC is involved in the action of cardiac κ-receptor stimulation, the PKC activity upon κ-receptor stimulation needs to be determined. The action of PKC on the SR and the exact site(s) of action of PKC in the AC/cyclic AMP pathway also warrants further study.

This study was supported by grants from Research Grant Council, Hong Kong. We thank Dr I. Bruce for critical review of the manuscript and Mr C.P. Mok for technical assistance. H.X.W. was supported by a post-doctoral fellowship of the University of Hong Kong (HK). J.S.B. was supported by a research assistantship of the Faculty of Medicine, HKU. W.M.Z. was on leave from Liu Hua Qiao Hospital, Guangzhou, China.

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(Received December 1, 1997
Revised February 20, 1998
Accepted February 26, 1998)