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Signaling pathway mediated by κ-opioid receptor is impaired in cardiac hypertrophy

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KEY WORDS cardiomegaly; kappa opioid receptors; protein kinase C; calcium; sodium-hydrogen antiporter

ABSTRACT

AIM: The responses of the intracellular calcium ($[Ca^{2+}]_i$) and the intracellular pH (pH_i) to κ-opioid receptor stimulation were determined in the single right ventricular myocytes isolated from the hearts of chronically hypoxic rats which exhibited right ventricular hypertrophy (RVH). **METHODS**: With the spectrofluorometric method, the electrically-induced $[Ca^{2+}]_i$ transient and pH_i were measured in myocytes loaded with fura-2 and BCECF [2′, 7′-bis-(2-carboxyethyl)-5-(and 6)-carboxyfluorscein], respectively. **RESULTS**: U50,488H, a selective κ-opioid agonist decreased the electrically-induced $[Ca^{2+}]_i$ transient and increased the pH_i. The effect of U50,488H was mediated by protein kinase C (PKC). In the RVH, the effect of U50,488H on the $[Ca^{2+}]_i$ transient and the pH_i were significantly attenuated. In parallel, 4-phorbol 12-myristate 13-acetate (PMA), an activator of PKC, also decreased the $[Ca^{2+}]_i$ transient and increased the pH_i. In the RVH, the effects of PMA were blunted. The recovery of pH_i, which was blocked by ethylisopropyl-amiloride (EIPA), following an acid loading induced by washout of 10 mmol/L NH_4Cl exposing to the cells for 10 min was the same in the RVH and control myocytes. **CONCLUSION**: κ-Opioid receptor signaling was impaired in the cardiac hypertrophy due to a defect in the coupling of PKC signaling with its effector.

INTRODUCTION

κ-Opioid receptors[1] and κ-opioid peptides[2] are present in the heart. The signaling pathway of κ-opioid receptor has been well elucidated in the previous studies[3,4]. It has been demonstrated that κ-opioid receptor stimulated by κ-opioid agonist increases the intracellular $[Ca^{2+}]_i$ and decreases the electrically-induced $[Ca^{2+}]_i$ transient by depleting the sarcoplasmic reticulum (SR) of Ca^{2+} via phospholipase C (PLC)/IP_3 pathway[5] and by negatively modulating the effect of β-adrenergic receptor stimulation[6] via a cyclic AMP-dependent pathway[7]. More importantly, κ-opioid receptor stimulation also activates PLC/protein kinase C (PKC) pathway, which leads to the further depletion of the SR by inhibition of Ca^{2+} uptake[4,8], and leads to an increase in intracellular pH (pH_i) by stimulating the Na^+-H^+ exchange in the sarcolemma[3].

It has been shown that action of Na^+-H^+ exchange stimulated by angiotensin II was altered in the cardiac hypertrophy[9], suggesting an altered signaling pathway in a receptor stimulation in cardiac hypertrophy. These alterations of intracellular events suggest a potential for differing intracellular signal transduction pathways upon κ-opioid receptor stimulation in adult hypertrophied myocytes. However, very little information is available on changes in receptor systems signaling through the PLC/PKC pathway in cardiac hypertrophy[10] and the investigation on this signaling pathway in right ventricular hypertrophy (RVH) is still lacking.

The objective of this study was to test the hypothesis that the effects of κ-opioid receptor stimulation on $[Ca^{2+}]_i$ transient and pH_i in adult hypertrophied myocytes differ from those in adult normal myocytes. Therefore, we compared the effects of κ-opioid agonist, as well as PKC agonist on the electrically-induced $[Ca^{2+}]_i$ transient and pH_i in single right ventricular (RV) myocytes isolated from the hearts of age-matched normal rats and right ventricular hypertrophied rats subjected to chronic hypoxia for 4 weeks. We also determined the recovery

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of pH_i from the acidification induced by washout of NH_4Cl pulse in the absence and presence of blockage of Na^+-H^+ exchange.

MATERIALS AND METHODS

Chronic hypoxia and assessment of RVH Male Sprague-Dawley rats (Grade Ⅱ, from the animal unit of the University of Hong Kong with the license number 980008) weighing (~100 g) at the start of the experiment were randomly divided into two groups. One group of the rats was exposed to chronic hypoxia and the control was maintained in room air. All the rats were kept in the same room with the same light-dark cycle. For chronic hypoxia, rats were given inspired oxygen at (10 % O_2) in a 300-liter acrylic chamber. The hypoxic environment was established with the inflow of a mixture of room air and nitrogen, which was regulated by an oxygen analyzer (model 175518A; Gold Edition, Vacuum Med, USA)[11]. Carbon dioxide was absorbed by soda lime granule, and access humidity was removed by desiccator. Temperature was maintained at 19 – 21 ℃. The chamber was opened twice a week for about 1 h to clean the cages and replenish food and water. Rats were exposed to hypoxia for 4 weeks and started experiment quickly after removal from the chamber.

The rats were weighed, decapitated and the hearts were quickly removed. The heart weight was determined. The right ventricular (RV) weight was also determined following removal of the left ventricle and septum. The RVH was indicated by calculating the ratio of whole heart weight over body weight, and the ratio of RV weight over body weight.

Isolation of ventricular myocytes RV myocytes were isolated from the heart of male Sprague-Dawley rats, using a collagenase perfusion method described previously[12]. Immediately after decapitation, the heart was rapidly removed from the rat and perfused in a retrograde manner at a constant flow rate (10 mL/min) with oxygenated Joklik modified Eagle's medium (supplemented with $CaCl_2$ 1.25 mmol/L and HEPES 10 mmol/L, pH 7.2, at 37 ℃ 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 kU/L with 0.1 % bovine serum albumin (BSA). After 35 – 40 min of perfusion with medium containing collagenase, the atria were discarded and the RV tissue was dissociated by shaking in the same oxygenated collagenase-free solution for 5 min at 37 ℃. The ventricular tissue was cut into small pieces with a pair of scissors followed by stirring with a glass rod, 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 1 % 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 mmol/L in 40 min.

Measurement of $[Ca^{2+}]_i$ Right ventricular myocytes were incubated with fura-2/AM (acetoxymethyl) at 5 μmol/L in Joklik solution supplemented with $CaCl_2$ 1.25 mmol/L for 30 min. The unincorporated dye was removed by washing the cells twice in fresh incubation solution. The loaded cells were kept at room temperature (25 ℃) for 30 min before measurements of $[Ca^{2+}]_i$ to allow the fura-2/AM in the cytosol to de-esterify. Loading with a low concentration of fura-2/AM and at a relatively low temperature of 25 ℃ was to minimize the effects of compartmentalization of the esters[13].

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, USA). The myocytes were perfused with a Krebs bicarbonate buffer containing (in mmol/L) 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, pH 7.4. The myocytes selected for the study were rode shaped and quiescent 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 (F_{340}) and 380 nm (F_{380}) excitation wavelengths were stored in a computer for data processing and analysis. The fluorescence (F_{340}/F_{380}) ratio was used to represent $[Ca^{2+}]_i$ changes in the myocytes.

For the measurement of electrically-induced $[Ca^{2+}]_i$ transient, the ventricular myocyte was superfused with Krebs solution, the cell was then electrically stimulated before administration of U50,488H or PMA. In experi-

ments involving nor-BNI, the drug was administered at 5 min before U50,488H. In experiments involving calphostin C, the drug was administered at 10 min before U50,488H or PMA. The electrically-induced $[Ca^{2+}]_i$ transient was recorded at about 10 min after administration of U50,488H or PMA.

Measurement of pH_i The pH_i was measured in the single myocyte as described previously[14]. The apparatus and optical arrangement used for the measurement of fluorescent light emission and preparation procedure were similar to those described in Measurement of $[Ca^{2+}]_i$ in the previous section except that the cells were loaded with the membrane-permeable acetoxymethyl ester, BCECF/AM [2′,7′-bis-(2-carboxyethyl)-5-(and 6)-carboxyfluorscein/acetoxymethyl ester] as the fluorescence indicator at 5 μmol/L for 30 min. The loaded cells were transferred to the stage of an inverted microscope in the superfusion chamber at room temperature. Myocytes were continuously superfused with a oxygenated HEPES – buffered normal Tyrode solution of the following composition (in mmol/L): NaCl 137, KCl 3.7, $MgCl_2$ 0.5, HEPES (free acid) 4.0, $CaCl_2$ 1.25, glucose 11 with a final pH of 7.4. Myocytes were superfused with this HEPES-buffered solution in order to eliminate effects of Cl^-/HCO_3^- exchange and Na^+/HCO_3^- exchange on pH_i[15]. The pH dependent signal of BCECF was obtained by illuminating at 490 and 435 nm and the fluorescent emission wavelength was measured at 520 nmol/L. The ratio of F490 over F435 was used to represent the pH_i.

At the end of each experiment, the calibration of BCECF signals was performed. The intracellular pH was set to the extracellular pH with 10 μmol/L nigericin in the calibration solution (HEPES 12, KCl 140, $MgCl_2$ 1, glucose 11 mmol/L). The extracellular pH was adjusted to 8,7,6,5 respectively using KOH or HCl.

For the measurement of pH_i, the ventricular myocyte was superfused with HEPES-buffered tyrode solution, U50,488H 20 μmol/L was then administered. In experiments involving nor-BNI, the drug was administered at 5 min before U50,488H. In experiments involving calphostin C or EIPA, both the drugs were administered at 10 min before U50,488H respectively. In experiments involving NH_4Cl, NH_4Cl 10 mmol/L was administered for 10 min. In experiments involving EIPA, the drug was administered at 10 min before washout of NH_4Cl.

Drugs and chemicals U50,488H (trans-3,4-dichloro-*N*-methyl-[2-(1-pyrrolidinyl) cyclohexy] benzeacet-amidel), PMA (4-phorbol 12-myristate 13-acetate), fura-2/AM, type I collagenase, nigericin and calphostin C were purchased from Sigma Chemicals Co (USA). Nor-BNI was purchased from Tocris Cookson Ltd (UK). BCECF-AM and EIPA (ethylisopropyl-amiloride) were purchased from RBI (USA).

Fura-2/AM, BCECF-AM, PMA, calphostin C, and EIPA were dissolved in dimethyl sulphoxide (Me_2SO), and nigericin was dissolved in the ethanol and the rest were dissolved in distilled water.

U50,488H at the dose range of 10 – 30 μmol/L was administered for 10 min as preliminary studies showed that the effects of the opioid were obvious at 2 – 3 min and reached maximum before 10 min. The dose range used in the present study has been shown to increase the $[Ca^{2+}]_i$ and IP_3, effects antagonized by nor-BNI at 1 – 5 μmol/L[6,16], which itself had no effect on any of the preparations studied. The concentrations of the PKC agonist and antagonist used were based on previous studies[17]. In a preliminary experiment, EIPA 10 μmol/L did not alter the autofluorescence of cells at the BCECF excitation wavelength as previously reported[18]. The final concentration of Me_2SO was 0.1 % and at this concentration Me_2SO had no effect on either $[Ca^{2+}]_i$ or pH_i[18].

Statistical analysis Values are expressed as $\bar{x} \pm s$. Paired Student's *t*-test was used to determine the difference between control and drug treatment groups. Unpaired Student's t test was employed to determine the difference among groups. Significance level was set at $P < 0.05$.

RESULTS

Characteristics of animal groups The body weight in the hypertrophied group was less than that in the age-matched normal group. The RVH was evidenced by the significantly increased ratios of heart weight over body weight and RV weight over body weight (Tab 1).

The peak systolic $[Ca^{2+}]_i$ transient indicated by fura-2 fluorescence ratio was lesser in the hypertrophied myocytes than that in control myocytes, while the end diastolic $[Ca^{2+}]_i$ transient was similar in two groups of myocytes (Tab 1). The baseline of pH_i was also not different from each other.

Effects of U50, 488H and PMA on electrically-induced $[Ca^{2+}]_i$ transient In agreement with

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Tab 1. Characteristics of animal groups. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* **normaoxic rats.**

	Normoxic	Hypoxic
	$n = 30$	$n = 32$
BW (g)	302 ± 32	215 ± 39^c
HW (mg)	1 198 ± 319	1 117 ± 288
RVW (mg)	284 ± 33	349 ± 51^b
HW/BW (mg/g)	4.0 ± 0.6	5.5 ± 1.5^c
RVW/BW (mg/g)	0.94 ± 0.22	1.6 ± 0.4^c
LVW + septum (mg)	914 ± 262	825 ± 254
(LVW + septum)/BW (mg/g)	3.0 ± 1.4	3.8 ± 1.6
	$n = 30$	$n = 30$
Peak-systolic $[Ca^{2+}]_i$ (ratio)	1.50 ± 0.11	1.28 ± 0.11^c
End-diastolic $[Ca^{2+}]_i$ (ratio)	0.75 ± 0.05	0.76 ± 0.05
pH_i (HEPES buffer)	7.03 ± 0.16	7.04 ± 0.22
pH_i (bicarbonate buffer)	6.94 ± 0.11	6.96 ± 0.16

BW: body weight; HW: heart weight; RVW: right ventricular weight; LVW: left ventricular weight. The $[Ca^{2+}]_i$ and pH_i values are the same in the right and left ventricules.

previous studies[19], U50, 488H, a selective κ-opioid receptor agonist, at range of 10 – 30 μmol/L dose-dependently decreased the electrically-induced $[Ca^{2+}]_i$ transient. The effect was abolished in the presence of nor-BNI 5 μmol/L (Fig 1A), a selective κ-opioid receptor antagonist[20], indicating that the effect was mediated by the κ-opioid receptor. The effect of U50,488H 20 μmol/L was significantly attenuated by calphostin C 1 μmol/L (Fig 1B), indicating that effect of κ-opioid receptor stimulation was at least partly mediated by PKC[4].

It was also find that PMA, an activator of PKC, at range of 0.01 – 1 μmol/L also dose-dependently decreased the electrically-induced $[Ca^{2+}]_i$ transient. The effect was abolished by calphostin C 1 μmol/L (Fig 2), a specific PKC inhibitor[21]. Results indicated that the effect of PMA on $[Ca^{2+}]_i$ transient was mediated by PKC activation.

It was found that the effect of U50, 488H at 20 μmol/L was significantly attenuated in the RV hypertrophied myocytes (Fig 3). In parallel and more interestingly, the effect of PMA at 0.1 μmol/L was also absent in the RV hypertrophied myocytes (Fig 4). These results implied that downstream of PKC in the cardiac κ-Opioid receptor-mediated signaling pathway was impaired in the RV hypertrophied myocytes.

Effects of U50, 488H and PMA on pH_i To investigate whether the regulation of pH_i by κ-opioid receptor is changed in cardiac hypertrophy, we

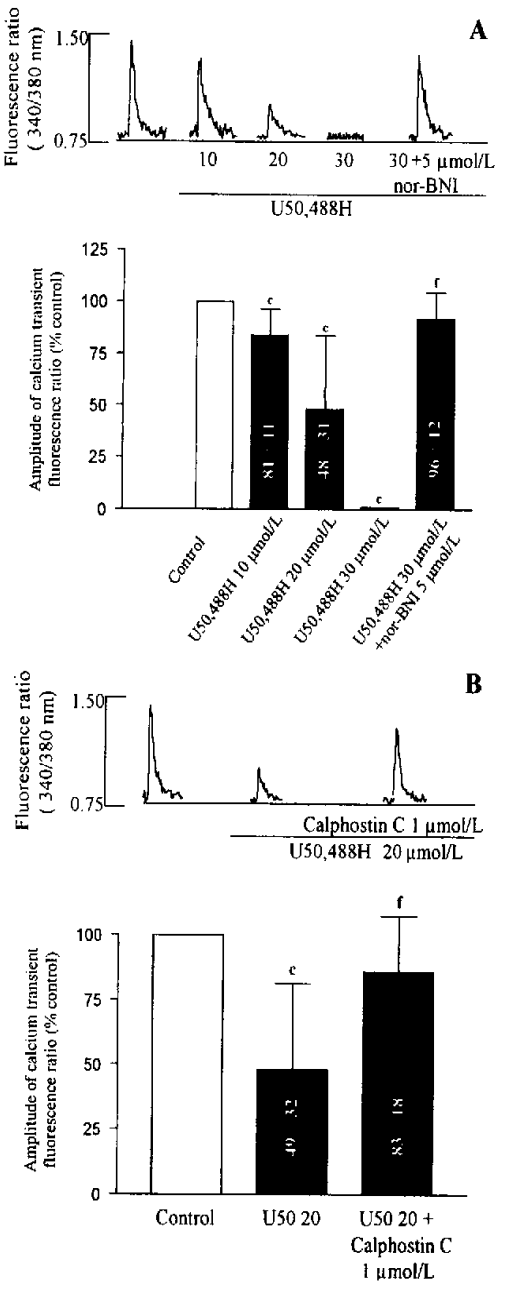

Fig 1. Effects of κ-opioid receptor stimulated with U50,488H on electrically-induced $[Ca^{2+}]_i$ transient in the normal single ventricular myocyte. A) Representative tracings (upper) and group results (lower) showing the dose-related effects of U50, 488H on the electrically-induced $[Ca^{2+}]_i$ transient in the presence and absence of nor-BNI 5 μmol/L. B) Representative tracings (upper) and group results (lower) showing the effects of U50,488H 20 μmol/L on the electrically-induced $[Ca^{2+}]_i$ transient in the presence and absence of calphostin C 1 μmol/L. n = 12 in each group in A. $n = 20$ in each group in B. $\bar{x} \pm s$. $^cP < 0.01$ *vs* corresponding control. $^fP < 0.01$ *vs* corresponding control with U50,488H.

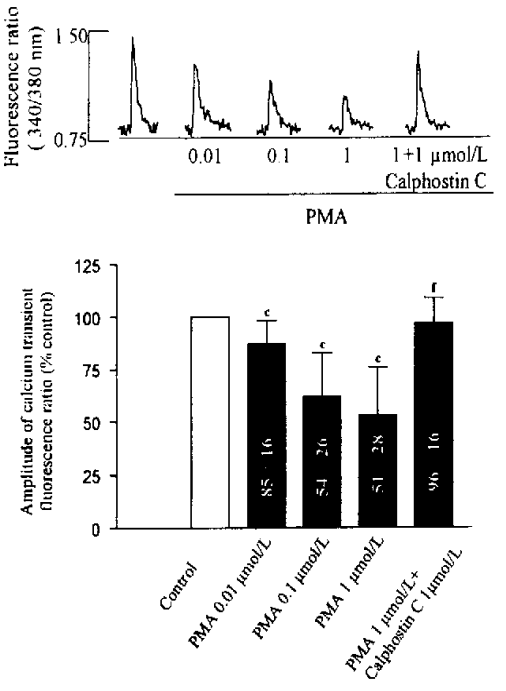

Fig 2. Effects of PKC activated with PMA on electrically-induced $[Ca^{2+}]_i$ transient in the normal single ventricular myocyte. Representative tracings (upper) and group results (lower) showing the effects of PMA 0.01 – 1 μmol/L on the electrically-induced $[Ca^{2+}]_i$ transient in the presence and absence of calphostin C 1 μmol/L. $n = 12$ in each group. $\bar{x} \pm s$. $^cP < 0.01$ *vs* corresponding control. $^fP < 0.01$ *vs* corresponding control with PMA.

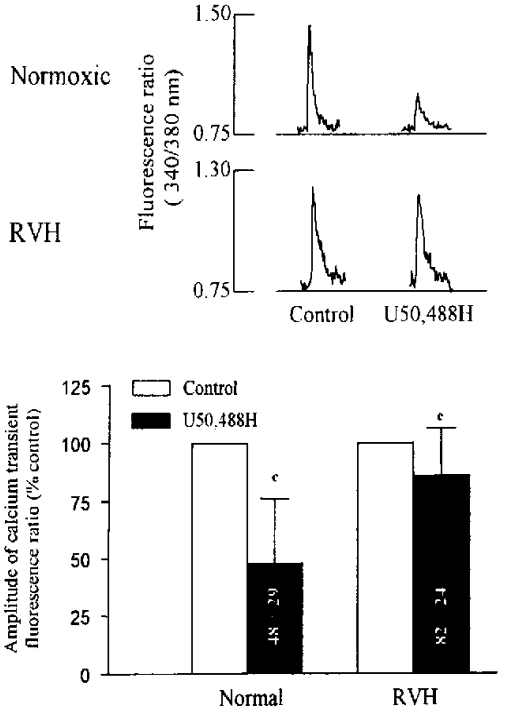

Fig 3. Effects of κ-opioid receptor stimulated with U50,488H on the electrically-induced $[Ca^{2+}]_i$ transient in the single right ventricular hypertrophied myocyte. Representative tracings (upper) and group results (lower) showing the effects of U50,488H 20 μmol/L on the electrically-induced $[Ca^{2+}]_i$ transient. $n = 18$ in each group. $\bar{x} \pm s$. $^cP < 0.01$ *vs* corresponding control. $^cP < 0.05$ *vs* corresponding control with U50,488H.

determined the pH_i upon κ-opioid receptor stimulation in two groups of myocytes. It was found that U50,488H at 20 μmol/L increased the pH_i (Fig 5A), the effect was respectively abolished in the presence of nor-BNI 5 μmol/L, calphostin C 1 μmol/L, and EIPA 10 μmol/L (Fig 5A), the latter is a potent Na^+-H^+ exchange blocker. The results indicated that κ-opioid receptor stimulation induced an alkinilization of the myocyte by activating $Na^+ - H^+$ exchange via PKC. In parallel, activation of PKC by PMA 0.1 μM also increased the pH_i (Fig 6A), which mimicked effect of U50,488H 20 μmol/L. The effect of PMA 0.1 μmol/L was completely inhibited by calphostin C 1 μmol/L (Fig 6A).

Correlating with the above phenomenon, it was found that both of the alkinilization induced by κ-opioid receptor stimulation (Fig 5A and B) and PKC activation (Fig 6A and B) were not seen in the RV hypertrophied myocytes. This was also a demonstration for the impairment in PKC-mediated signaling pathway in the cardiac hypertrophy.

Effects of NH_4Cl pulse on pH_i in the absence and presence of EIPAi To elucidate whether PKC coupling or the end effector of PKC was involved in the impairment, we determined the function of Na^+-H^+ exchange, an end effector of PKC, by exposing the cells to NH_4Cl pulse which leads to intracellular acidosis and measuring the recovery of pH_i. Previous studies have clarified that the initial rate of recovery from this intracellular acidosis depends predominantly on forward Na^+-H^+ exchange[22]. The effects of a NH_4Cl pulse in the presence and absence of EIPA 10 μmol/L was examined by exposure and washout of HEPES-buffered solution containing 10 mmol/L NH_4Cl prepared by equimolar replacement of NaCl with NH_4Cl. In agreement with previous studies[23], the abrupt exposure

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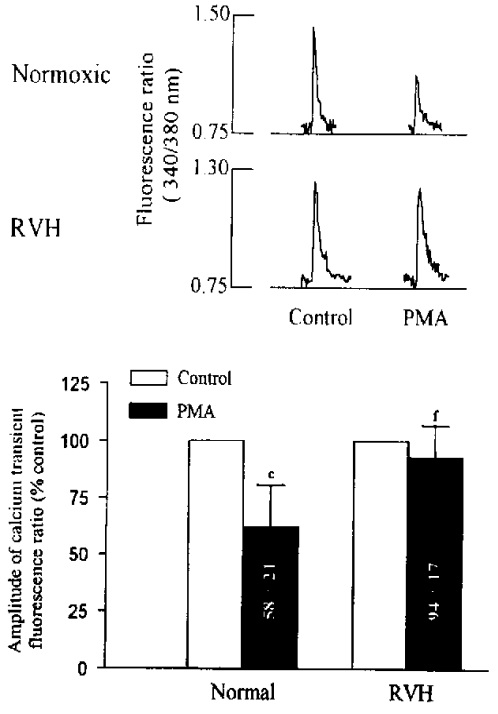

Fig 4. Effects of PKC activated with PMA on the electrically-induced $[Ca^{2+}]_i$ transient in the single right ventricular hypertrophied myocyte. Representative tracings (upper) and group results (lower) showing the effects of PMA 0.1 μmol/L on the electrically-induced $[Ca^{2+}]_i$ transient. $n = 20$ in each group. $\bar{x} \pm s$. $^cP < 0.01$ *vs* corresponding control. $^fP < 0.01$ *vs* corresponding control with PMA.

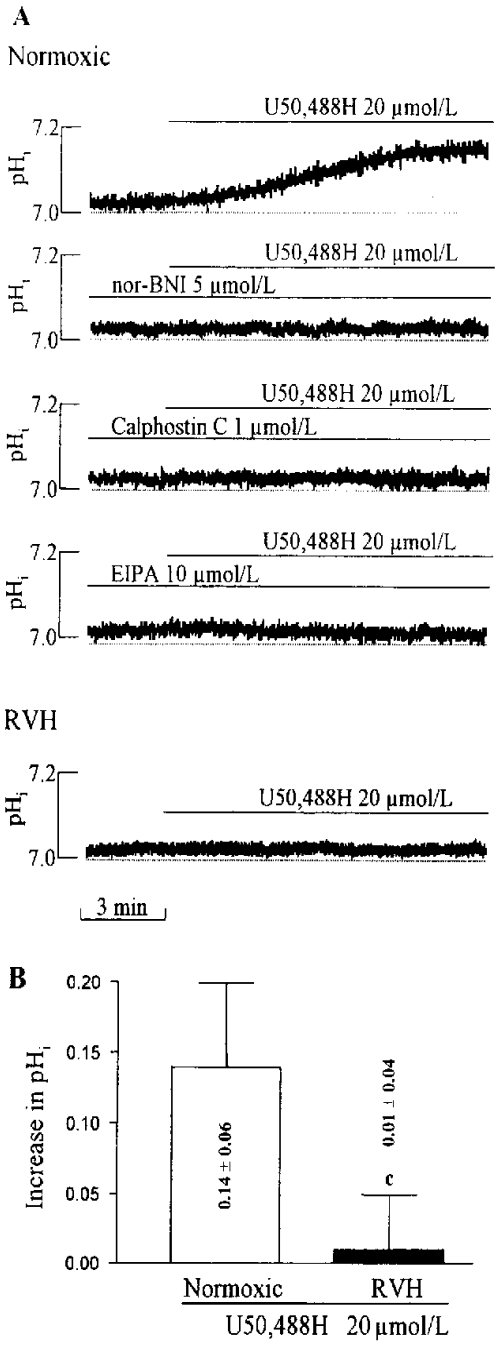

Fig 5. Effects of κ-opioid receptor stimulated with U50,488H on pH_i in the normal single ventricular myocyte and hypertrophied myocyte. A) Representative tracings showing the effects of 20 μmol/L U50,488H on the pH_i in the presence and absence of nor-BNI 5 μmol/L, calphostin C 1 μmol/L and EIPA 10 μmol/L. B) Group results showing the effects of 20 μmol/L U50,488H on the pH_i in normal and hypertrophied myocyte. The pH_i was recorded at about 10 min after administration of U50, 488H. $n = 10$ in two groups. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control.

to NH_4Cl initially increased pH_i (Fig 7A). Upon washout of extracellular NH_4Cl, intracellular acidosis was created, followed by recovery of pH_i, this recovery of pH_i was blocked in the presence of EIPA 10 μmol/L (data not shown). It was found that the time course recovery of pH_i was in a similar manner in the two groups of myocytes (Fig 7A and B), indicating that the PKC coupling, not the end effector (Na^+-H^+ exchange) was impaired in cardiac hypertrophy.

DISCUSSION

The most important finding in the present study is that the coupling of PKC with the end effectors upon κ-opioid receptor stimulation is impaired in the cardiac hypertrophy. We also for the first time demonstrated that signaling pathway mediated by κ-opioid receptor was altered in the right ventricular hypertrophied myocytes, which is based on the following three observations.

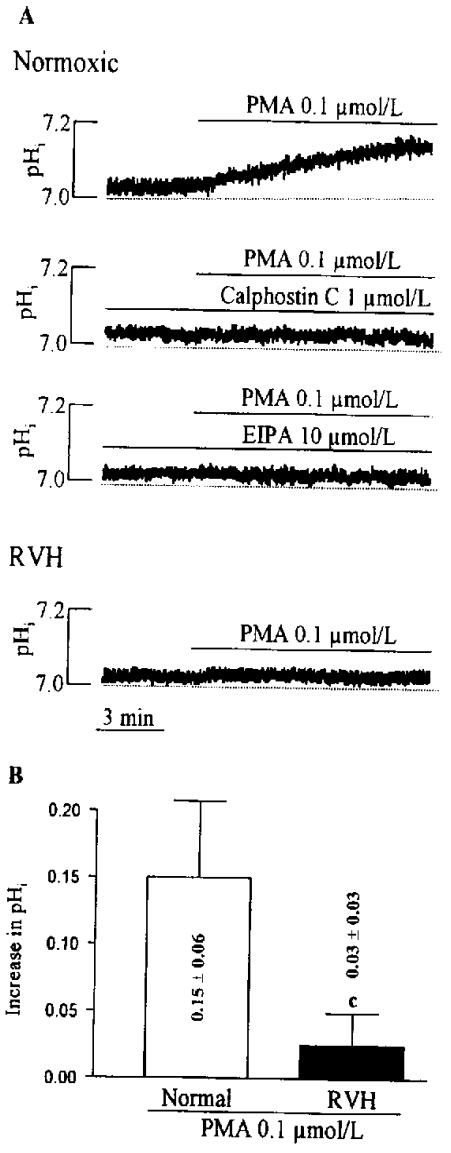

Fig 6. Effects of PKC activated with PMA on pH_i in the normal single ventricular myocyte and hypertrophied myocyte. A) Representative tracings showing the effects of PMA 0.1 μmol/L on the pH_i in the presence and absence of calphostin C 1 μmol/L and EIPA 10 μmol/L. B) Group results showing the effects of PMA 0.1 μmol/L on the pH_i in normal and hypertrophied myocyte. $n = 10$ in each group. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control.

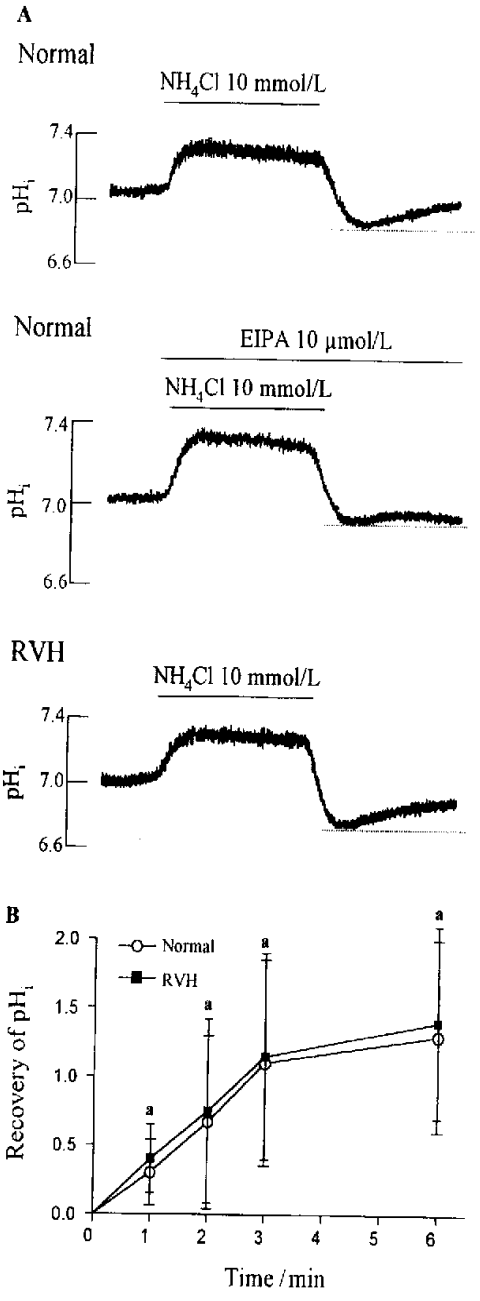

Fig 7. Effects of Na^+-H^+ exchange stimulated with washout of NH_4Cl pulse on pH_i responses in the normal single ventricular myocyte and hypertrophied myocyte. A) Representative tracings showing the effects of exposure and washout of NH_4Cl 10 mmol/L on the pH_i in the presence and absence of EIPA 10 μmol/L in normal and hypertrophied myocyte. B) Group results showing the time course of pH_i recovery from intracellular acidosis elicited by washout of a NH_4Cl 10 mmol/L pulse in normal and hypertrophied myocyte. The recovery value for 6 min after washout of NH_4Cl is shown. $n = 15$ in each group. $\bar{x} \pm s$. $^aP > 0.05$ *vs* corresponding control.

firstly, activation of PKC induced by κ-opioid receptor stimulation with the κ-opioid receptor agonist mediated a decreased electrically-induced $[Ca^{2+}]_i$ transient and an increased pH_i in the normal group of myocytes. These effects were all blunted in the adult hypertrophied

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myocytes. Secondly, correlating with above phenomenon, direct PKC activation induced by PKC agonist also decreased the electrically-induced $[Ca^{2+}]_i$ transient and increased pH_i in the normal group of myocytes. These effects were also blunted in the adult hypertrophied myocytes. Thirdly, the function of end effector of PKC, such as Na^+-H^+ exchange was not changed in the hypertrophied myocytes. These results clearly indicated that signaling mechanism of the impairment in the cardiac κ-opioid receptor involved the PKC coupling.

It is confirmatory to find that the extent in $[Ca^{2+}]_i$ or pH_i, both downstream of PKC, in response to κ-opioid receptor stimulation was disparate in normal myocytes. That is, the $[Ca^{2+}]_i$ response to κ-opioid receptor stimulation was attenuated while the pH_i response was abolished in the presence of calphostin C, a highly specific PKC inhibitor. This could be due to the fact that PLC/PKC pathway partly affects the $[Ca^{2+}]_i$ homeostasis upon κ-opioid receptor stimulation[4], while it was fully responsible for the pH_i response via stimulating of Na^+-H^+ exchange[3]. Our further paralleling experiment in this study showed that direct PKC activation with PMA fully accounted for the response of $[Ca^{2+}]_i$ as well as pH_i, providing the direct evidence that PKC activation affects the $[Ca^{2+}]_i$ homeostasis. It has been demonstrated that PKC activation with PMA has a negative inotropic effect mediated by the decrease in $[Ca^{2+}]_i$ transient in adult rat cardiac myocytes[17]. It is well known that electrically induced $[Ca^{2+}]_i$ transient results from an influx of Ca^{2+} upon membrane depolarization, which triggers a sudden release of Ca^{2+} from the sarcoplasmic reticulum via a Ca^{2+}-induced Ca^{2+} release mechanism. So, in the presence of electrical stimulation, the negative inotropic effect of PKC activation may raise an explanation for decrease in electrically-induced $[Ca^{2+}]_i$ transient via PKC upon κ-opioid receptor stimulation.

It is well known that chronic hypoxia results in the pulmonary hypertension, which is responsible for the development of RVH. It seems to be interesting to find that the baseline amplitude of electrically-induced $[Ca^{2+}]_i$ transient was less in hypertrophied myocytes of rat subjected to chronic hypoxia for 4 weeks. This would lead us to consider that 4 weeks chronic hypoxia may lead the myocardium to approach in the decompensated period. Regarding the exact transition process from compensated hypertrophy to heart failure and underlying mechanism, further experiment on time course study is needed.

It has been demonstrated that gene expression of Na^+-H^+ exchange increases in the early period of time before hypertrophy formation, the phenomenon remains for 2 weeks and plays a role in the course of development of hypertrophy[24]. In fact, the increased gene expression may not be necessarily to be followed by an increased protein level or its function. In the present study, the RVH has developed after 4 weeks of chronic hypoxia, we found that the baseline of pH_i was not changed in the hypertrophied myocytes compared with controls. Our further study also showed that the pH_i recovery from the acidosis induced by washout of NH_4Cl pulse was still similar in two groups of myocytes, indicating that the function of Na^+-H^+ exchange is not changed in the cardiac hypertrophy. Together with the observation of absent pH_i response to PKC activation by PKC agonist or κ-opioid receptor agonist, this result further supports that coupling of PKC to Na^+-H^+ exchange, an end effector of PKC, was impaired in cardiac hypertrophy.

In conclusion, the present study for the first time demonstrated that κ-opioid receptor signaling through the PLC/PKC pathway was impaired in cardiac hypertrophy. Changes of $[Ca^{2+}]_i$ and pH_i in response to PKC activation with PKC agonist were blunted, which mimicked those in response to κ-opioid receptor stimulation in hypertrophied myocytes, suggesting a defect in PKC coupling with effector in cardiac hypertrophy.

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心肌肥厚时 κ 阿片受体介导的信号通路受损

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关键词 心肥大; κ 阿片受体; 蛋白激酶 C; 钙; Na^+-H^+交换

目的: 分离正常及慢性缺氧性右心肥厚的心室肌细胞, 观察细胞内$[Ca^{2+}]_i$及细胞内 pH_i 对心肌 κ-阿片受体激动后的反应. **方法**: 以 fura-2 和 BCECF 分别为$[Ca^{2+}]_i$和 pH_i 的指示剂, 用光谱荧光法测定电刺激引起的细胞内$[Ca^{2+}]_i$瞬变及 pH_i. **结果**: κ 阿片受体选择性激动剂 U50,488H 可降低电刺激引起的$[Ca^{2+}]_i$瞬变和增加 pH_i, 该作用是由蛋白激酶 C (PKC)所介导. 在肥厚的心室肌细胞, U50,488H 的上述作用显著减弱. 与此相对应, PKC 的激动剂 PMA 引起的$[Ca^{2+}]_i$瞬变降低和 pH_i 的增加作用在肥厚的心室肌细胞亦消失. 用 NH_4Cl 法观察 Na^+-H^+交换器的功能显示其在肥厚的心室肌细胞无明显改变. **结论**: 心肌肥厚时 κ-阿片受体介导的信号通路受损, 受损部位发生在 PKC 与效应器之间.

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