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Alterations in protein kinase A and different protein kinase C isoforms in the heart during morphine withdrawal

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Abstract

The present study was designed to investigate the possible changes of protein kinase A (PKA) and different isoforms of protein kinase C (PKC): PKC α, PKC δ and PKC ζ after naloxone induced morphine withdrawal in the heart. Male rats were implanted with placebo (naïve) or morphine (tolerant/dependent) pellets for 7 days. On day 8 rats received saline s.c. or naloxone (5 mg/kg s.c.). The protein levels of PKA, PKC δ and PKC ζ were significantly up-regulated in the heart from morphine withdrawal rats. By contrast, morphine withdrawal induced down-regulation of PKC α. These results suggest that both PKA and PKC may be involved in the cardiac adaptive changes observed during morphine withdrawal.

Keywords: Heart; Morphine withdrawal; Protein kinase A; Protein kinase C

1. Introduction

Protein kinase A (PKA) and protein kinase C (PKC) play a central role in transducing signal transduction and potentiating intracellular cross talk by phosphorylating diverse substrates, including cell-surface receptors, enzymes and transcription factors. Both protein kinases are expressed in cardiac muscle (for review see Keef et al., 2001). The PKC family is comprised of at least 12 different forms (Hayashi et al., 1999). Based on their structures and ability to bind with the cofactors, these isozymes have been grouped into three subclasses: i) the conventional PKC comprises α, βI, βII and γ isozymes that are activated by Ca^{2+}, diacylglycerol (DAG) and phosphatidyl-serina (PS); ii) the novel PKC isozymes consisting of δ, ε, η and Θ isozymes that do not respond to Ca^{2+}, but are activated by PS and DAG; iii) the atypical isozymes consisting of ζ and τ/λ that are unresponsive to Ca^{2+}, DAG and phorbol esters, but are also activated by PS. Mammalian hearts have been found to coexpress a number of PKC isozymes, including α, βI, βII, δ, ε, and ζ (Naruse and King, 2000).

Accumulating evidence has shown that the cellular and molecular adaptation following long-term opioid exposure results from the phosphorylation of opioid receptor protein, their coupled G proteins, and several related effector proteins. The enzymes producing these changes include second messenger-dependent protein kinases (PKC), cyclic AMP-dependent protein kinase (PKA), Ca^{2+}/calmodulin-dependent protein kinase II (CAMKII), G protein-coupled receptor kinases and mitogen-activated protein kinases (MAPKs), which play important roles in the regulation of opioid signal transduction (for review see Liu and Anand, 2001). Alterations in both PKA and PKC pathways have been suggested as one of the molecular mechanism of opioid tolerance and dependence (Nestler and Aghajanian, 1997). Although the μ opioid receptor is negatively coupled to the adenylate cyclase/cAMP-dependent PKA pathway upon acute stimulation (Childers, 1991), both the PKA and PKC pathways are up-regulated in several brain areas with chronic morphine treatment (Nestler, 1992; Tokuyama et al., 1995). Previous studies in our laboratory have demonstrated that naloxone administration to morphine-dependent rats leads to an enhancement of cAMP levels in the heart (Milanés et al., 2000). In addition, chronic inhibition of PKA with the selective PKA

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inhibitor, HA-1004 significantly blocks the enhancement of noradrenaline turnover during morphine withdrawal in both the right and left ventricle (Martínez et al., 2003). Studies have also focused on the role of PKC in tolerance and dependence. Acute administration of PKC inhibitors was not able to reverse morphine dependence (Smith et al., 2002). In addition, the chronic inhibition of PKC with calphostin C did not modify the increase in the noradrenaline turnover observed during morphine withdrawal in the heart (Martínez et al., 2003). In contrast, it has been shown that PKC inhibitors prevent the development of opioid physical dependence (Fundytus and Coderre, 1996).

Although the involvement of PKA and PKC pathways in the morphine dependence have been reported, not enough data are available on the characteristic and functional disturbances of the heart protein kinases after chronic morphine treatment and upon drug withdrawal. Therefore, the purpose of the present study was to determine the possible changes in the expression of PKA and PKC isozymes (α, δ, and ζ) after naloxone precipitated morphine withdrawal in the heart.

2. Material and methods

Male Sprague–Dawley rats (220–240 g at the start of the experiments) were housed four to five per cage under a 12 h light/dark cycle (L:8:00–20:00 h) in a room with controlled temperature (22±2 °C) and humidity (50±10%) and food and water available *ad libitum*. Animals were pre-handled for several days preceding the experiment to minimize stress, as previously described (Laorden et al., 2000). All surgical and experimental procedures were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the local Committee.

2.1. Experimental procedure

Rats were rendered tolerant/dependent on morphine by s. c. implantation of morphine base pellets (75 mg): one on day 1, two on day 3 and three on day 5, under light ether

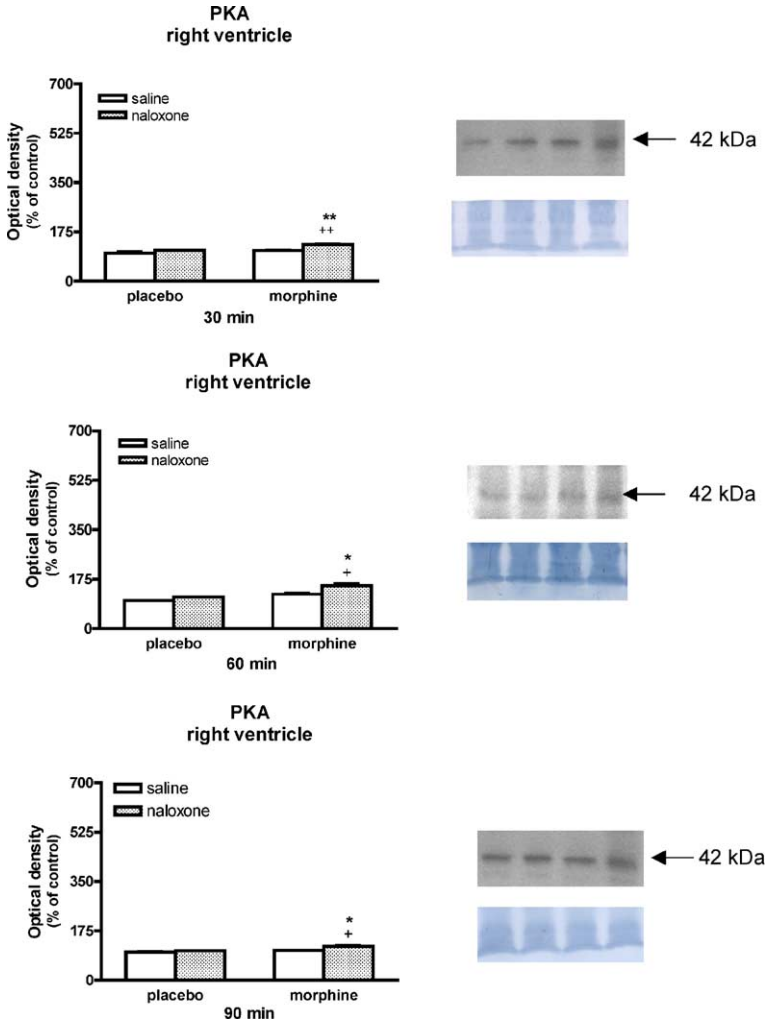

Fig. 1. Western blotting analysis of protein kinase (PKA) immunoreactivity levels in the right ventricle in different experimental groups. Animals were decapitated 30 min, 60 min or 90 min after saline or naloxone injection. The immunoreactivity corresponding to PKA is expressed as a percentage of that in the control group (placebo+saline; defined as 100% value). Data are means±S.E.M., $n=4$–6. *$P<0.05$, **$P<0.01$ versus morphine+saline; +$P<0.05$ versus placebo+naloxone. Right panels: representative band from autoradiograms at the known apparent molecular weight for PKA. Similar loading and transfer were ascertained by cutting the lower portion of the blot and staining for total protein with Amido Black.

anaesthesia. Control animals were implanted with placebo pellets containing lactose instead of morphine on the same time schedule. These procedures have repeatedly been shown to induce both tolerance and dependence as measured behaviourally and biochemically (Rabadán et al., 1998; Milanés and Laorden, 2000; Milanés et al., 2000). On day 8 the animals pretreated with morphine or placebo pellets were injected with saline s.c. or naloxone (5 mg/kg s.c.). Withdrawal signs were observed before and after the administration of the opioid antagonist or saline. The weight gain of the rats was checked during treatment to ensure that the morphine was liberated correctly from the pellets because it is known that chronic morphine treatment induces a decrease in body weight gain due to lower caloric intake (Berhow et al., 1995). In addition, body weight loss was determined as the difference between the weight determined immediately before saline or naloxone injection and a second determination made 30, 60 and 90 min later.

2.2. Tissue preparation for Western blotting analyses

Animals were killed by decapitation under light ether anaesthesia 30, 60 or 90 min after administration of naloxone or saline. The hearts were rapidly removed, and the right and left ventricles were dissected, fresh-frozen, and stored immediately at −80 °C until use.

Samples were placed in homogenization buffer [phosphate buffered saline, 2% sodium dodecylsulfate (SDS) plus protease inhibitors, Boehringer Mannhein, Germany] and homogenized for 50 s prior to centrifugation at 1811.16 g for 20 min at 4 °C. The supernatant was boiled (5 min), and total protein concentrations were determined spectrophotometrically using the bicinchoninic acid method (Wiechelman et al., 1988).The optimal amount of protein to be loaded was determined in preliminary experiments by loading gels with increasing protein contents (25 to 100 μg) from samples of each experimental group. Equal amounts of protein (50 μg/lane) from each sample were loaded on a 10% sodium dodecyl sulfate-polyacrilamide

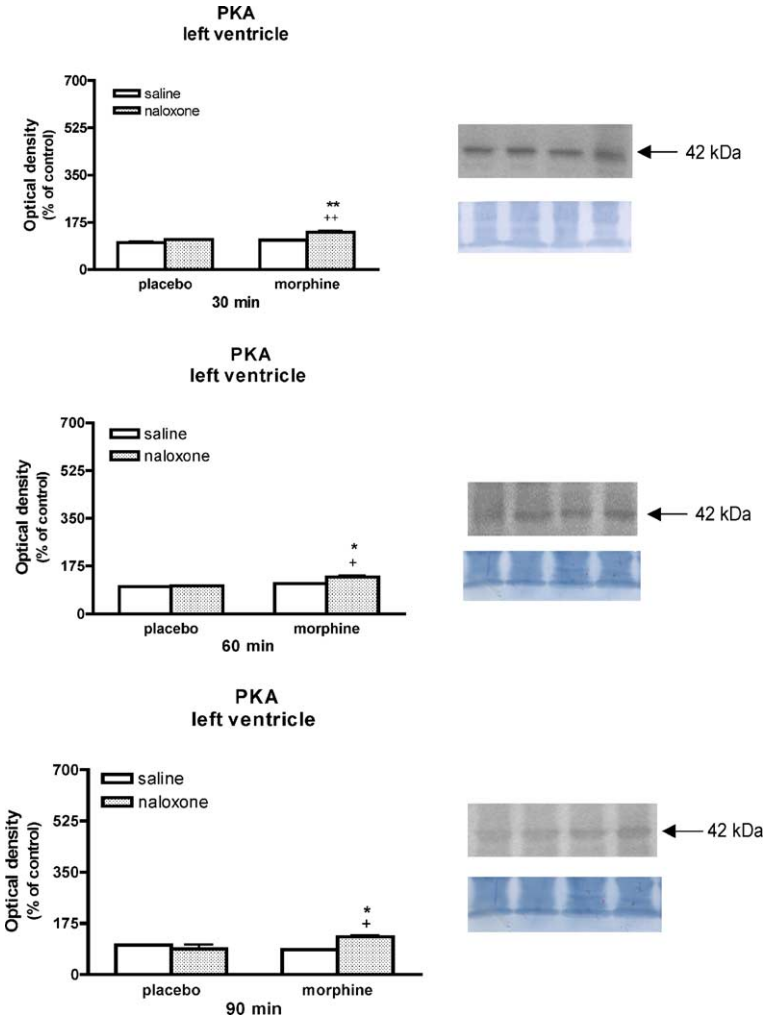

Fig. 2. Western blotting analysis of protein kinase (PKA) immunoreactivity levels in the left ventricle in different experimental groups. Animals were decapitated 30 min, 60 min or 90 min after saline or naloxone injection. The immunoreactivity corresponding to PKA is expressed as a percentage of that in the control group (placebo+saline; defined as 100% value). Data are means±S.E.M., $n=4$–6. *$P<0.05$, **$P<0.01$ versus morphine+saline; +$P<0.05$, ++$P<0.01$ versus placebo+naloxone. Right panels: representative band from autoradiograms at the known apparent molecular weight for PKA. Similar loading and transfer were ascertained by cutting the lower portion of the blot and staining for total protein with Amido Black.

gel (SDS-PAGE), electrophoresed, and transferred onto poly vinylidene difluoride (PVDF) membrane using a Mini Trans-Blot Electrophoresis Transfer Cell (Bio-Rad Lab., California, USA). Similar loading and transfer was ascertained by cutting the lower portion of the blot and staining for total protein with Amido Black. Non-specific binding of antibodies was prevented by incubating membranes in 1% bovine serum albumin (BSA) in tris-buffer saline tween (TBST: 10 mM Tris–HCl, pH 7.6, 150 mM NaCl, 0.05% Tween 20). The membranes were incubated overnight, at 4 °C, with the following primary antibodies: specific PKA catalytic subunit antibody (1 : 1000 dilution; sc-903, Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.). This antibody is directed against the α catalytic subunit of PKA; polyclonal primary anti-PKCα (1 : 1000 dilution; p4334, Sigma, Chemical Co., St Louis, MO, USA); polyclonal anti-PKCδ (1 : 5000 dilution; p8333, Sigma, Chemical Co., St Louis, MO, USA) and polyclonal anti-PKCζ (1 : 5000 dilution; p713, Sigma, Chemical Co., St Louis, MO, USA) in TBST with bovine serum albumin (BSA). After extensive washings with TBST, the membranes were incubated for 1 h, at room temperature, with peroxidase-labeled secondary antibodies (anti-rabbit sc-2004) for PKA and PKC isoforms, Santa Cruz at 1 : 5000 dilution. After washing, immunoreactiviy was detected with an enhanced chemiluminescence Western blot detection system (ECL, Amersham-Pharmacia-Biotechnology, Madrid, Spain) and visualised by Amersham Hyperfilm-ECL. After film scanning, the integrated optical density of the bands was estimated (Scion Image software, Scion Corporation, Maryland, USA), and normalised to the background values. Relative variations between the bands of the experimental samples and the control samples were calculated in the same image. Duplicate measurements in three or four different gels for each individual sample were performed. Measurements were in the linear range.

2.3. Drugs and chemicals

Pellets of morphine base (Alcaliber Laboratories, Madrid, Spain) or lactose were prepared by the Department of Pharmacy

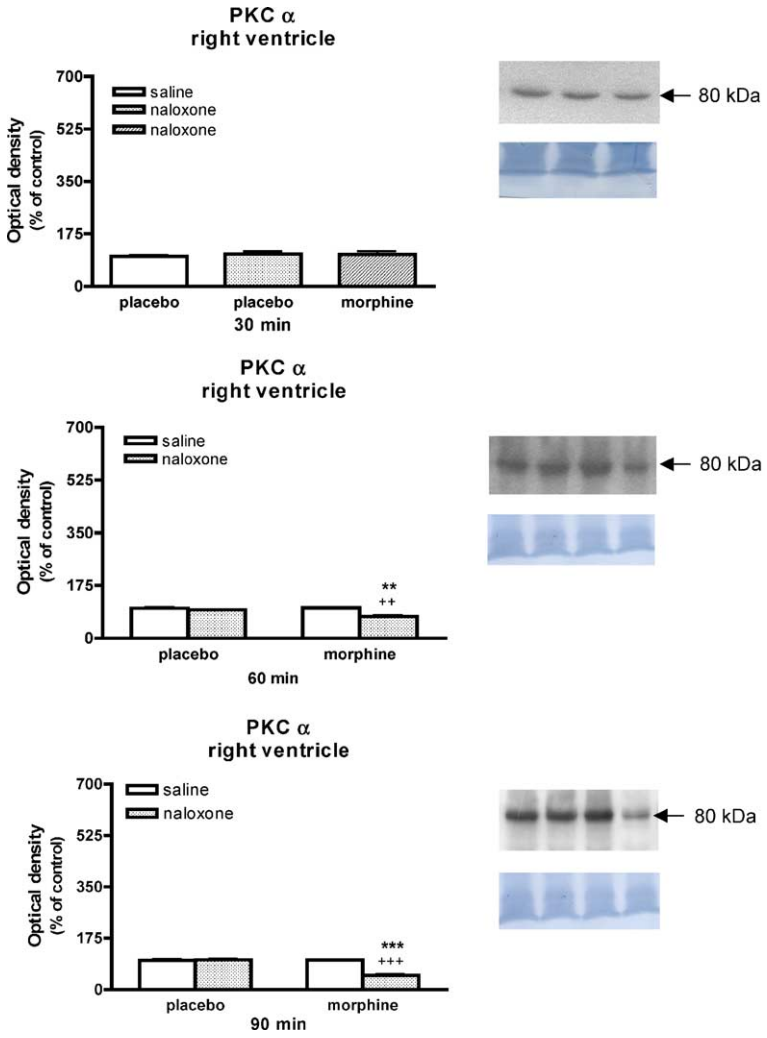

Fig. 3. Western blotting analysis of protein kinase (PKCα) immunoreactivity levels in the right ventricle in different experimental groups. Animals were decapitated 30 min, 60 min or 90 min after saline or naloxone injection. The immunoreactivity corresponding to PKCα is expressed as a percentage of that in the control group (placebo+saline; defined as 100% value). Data are means±S.E.M., $n=4$–6. $^{**}P<0.01$, $^{***}P<0.001$ versus morphine+saline; $^{++}P<0.01$, $^{+++}P<0.001$ versus placebo+naloxone. Right panels: representative band from autoradiograms at the known apparent molecular weight for PKCα. Similar loading and transfer were ascertained by cutting the lower portion of the blot and staining for total protein with Amido Black.

and Pharmaceutic Technology (School of Pharmacy, Granada, Spain); sodium dodecylsulphate, polyacrylamide gel and PVDF membrane were obtained from Bio-Rad Laboratory (Teknovas, Bilbao, Spain). Naloxone HCl and Western blot reagents were purchased from Sigma Chemical Co. (St Louis, MO, USA). Naloxone HCl was dissolved in sterile 0.9% NaCl (saline) and administered in volumes of 0.10 ml 100^{-1} g body weight.

2.4. Statistical analysis

The mean±S.E.M. was expressed for values obtained from a minimum of four rats per group. Data were analysed by analysis of variance (ANOVA) followed by the Newman–Keuls post-hoc test. Body weight gain and loss in naive and morphine-dependent rats was analysed by unpaired Student's *t*-test. Differences with a *P* value less than 0.05 were considered significant.

3. Results

Rats treated with morphine showed a significantly lower ($P<0.01$; *t*-test) body weight gain (22.37±2.21 g, $n=68$) than animals receiving placebo pellets (52.08±3.14 g, $n=72$). Administration of naloxone to control rats resulted in no significant changes in body weight when measured 30 (5.03 ±0.93 g, $n=16$), 60 (4.36±0.60, $n=16$) or 90 (2.38±0.55 g, $n=18$) min after drug injection, as compared to control rats receiving saline (1.66±0.36 g, $n=13$; 4.32±0.61 g, $n=17$; 3.69 ±1.60, respectively; *t*-test). However, chronic morphine-treated animals showed a significant weight loss ($P<0.001$; *t*-test) 30 (16.92±0.18 g, $n=15$), 60 (14.67±10.80 g, $n=16$) or 90 min (17.53±0.37 g, $n=20$) after naloxone injection when compared with the placebo-pelleted group also receiving naloxone. All morphine-dependent animals receiving naloxone demonstrated behaviours characteristic of opioid withdrawal, including jumping, wet-dog shakes, teeth chattering, ptosis, chromodacryorrhea and irritability.

3.1. Effects of morphine dependence on protein kinases levels in the heart

The influence of morphine dependence and withdrawal on the immunoreactivity of PKA and different isoforms of PKC was examined by Western-blot analysis in the right and left

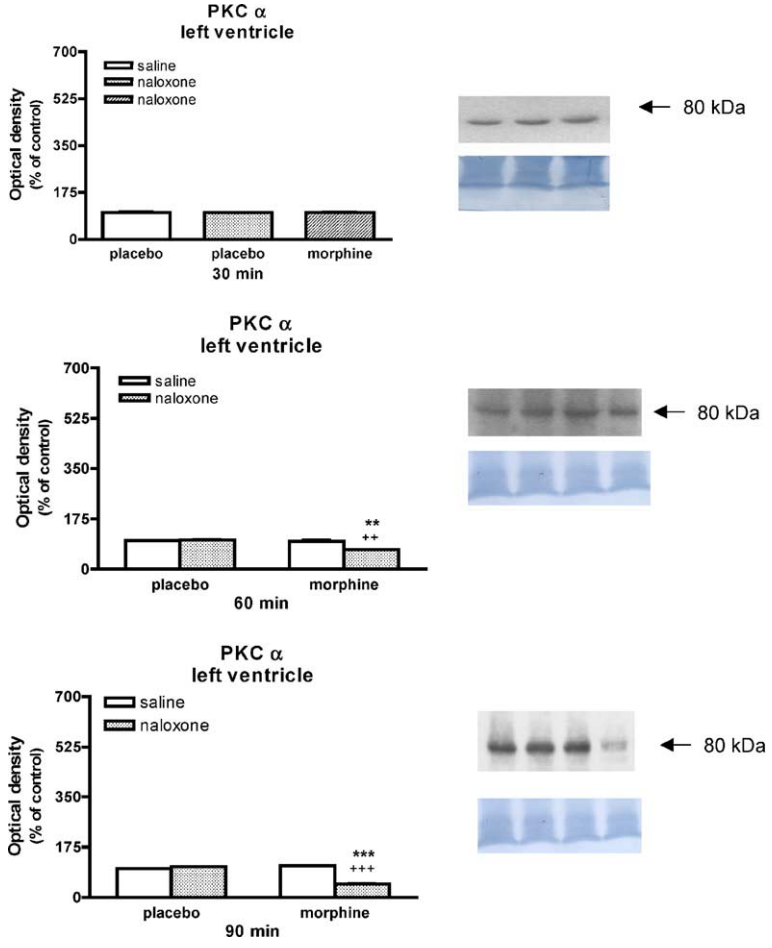

Fig. 4. Western blotting analysis of protein kinase (PKCα) immunoreactivity levels in the left ventricle in different experimental groups. Animals were decapitated 30 min, 60 min or 90 min after saline or naloxone injection. The immunoreactivity corresponding to PKCα is expressed as a percentage of that in the control group (placebo+saline; defined as 100% value). Data are means±S.E.M., $n=4$–6. $^{**}P<0.01$, $^{***}P<0.001$ versus morphine+saline; $^{++}P<0.01$, $^{+++}P<0.001$ versus placebo+naloxone. Right panels: representative band from autoradiograms at the known apparent molecular weight for PKCα. Similar loading and transfer were ascertained by cutting the lower portion of the blot and staining for total protein with Amido Black.

ventricle at different time points. The PKA was detected in a band located at ~42 kDa. PKA immunoreactivity was determined at 30, 60 or 90 min after saline or naloxone injection to naïve or morphine dependent rats. In control (placebo) animals injected with saline or naloxone only very low levels of PKA could be detected. There were no statistically significant differences between PKA levels in these two groups. In addition, PKA expression was low in morphine pelleted rats injected with saline. However, Western blot analysis revealed that naloxone administration to morphine dependent rats produced a significant induction of PKA catalytic subunit in right (Fig. 1) and left ventricle (Fig. 2) versus the dependent group treated with saline ($P<0.01$; $P<0.05$) or control rats receiving naloxone ($P<0.01$; $P<0.05$).

The influence of morphine withdrawal on the immunoreactivity of PKC isozymes (α, δ and ζ) was also examined in the right and left ventricle. The PKCα, PKC δ and PKCζ were detected in a band located at ~80 kDa. As shown in Figs. 3 and 4, PKCα expression was not modified 30 min after naloxone administration versus the group of rats treated with placebo pellets plus naloxone. However, there was a reduction of PKCα in the right and left ventricle 60 or 90 min after naloxone administration to morphine dependent rats versus morphine dependent rats injected with saline or placebo pelleted rats injected with naloxone ($P<0.01$; $P<0.001$) (Fig. 4). The administration of the opioid antagonist to control rats did not modify the level of PKCα compared with the corresponding control group receiving saline.

PKCδ and PKCζ immunoreactivity were not modified in the right or left ventricle 30 min after naloxone administration versus the group of rats treated with placebo pellets plus naloxone (Figs. 5–8). By contrast, as shown in Figs. 5 and 6, PKCδ levels were enhanced ($P<0.001$) 60 or 90 min after naloxone administration to morphine dependent rats versus morphine dependent rats injected with saline or control rats injected with naloxone. In animals treated with placebo for

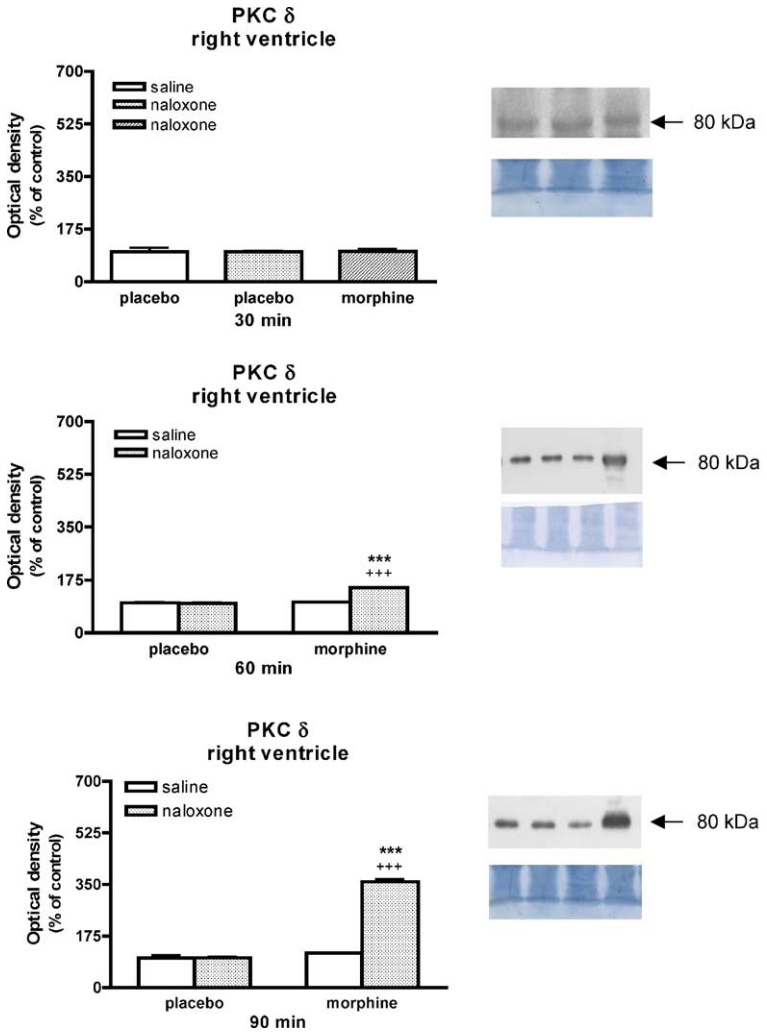

Fig. 5. Western blotting analysis of protein kinase (PKCδ) immunoreactivity levels in the right ventricle in different experimental groups. Animals were decapitated 30 min, 60 min or 90 min after saline or naloxone injection. The immunoreactivity corresponding to PKCδ is expressed as a percentage of that in the control group (placebo+saline; defined as 100% value). Data are means±S.E.M., $n=4$–6. ***$P<0.001$ versus morphine+saline; +++$P<0.001$ versus placebo+naloxone. Right panels: representative band from autoradiograms at the known apparent molecular weight for PKCδ. Similar loading and transfer were ascertained by cutting the lower portion of the blot and staining for total protein with Amido Black.

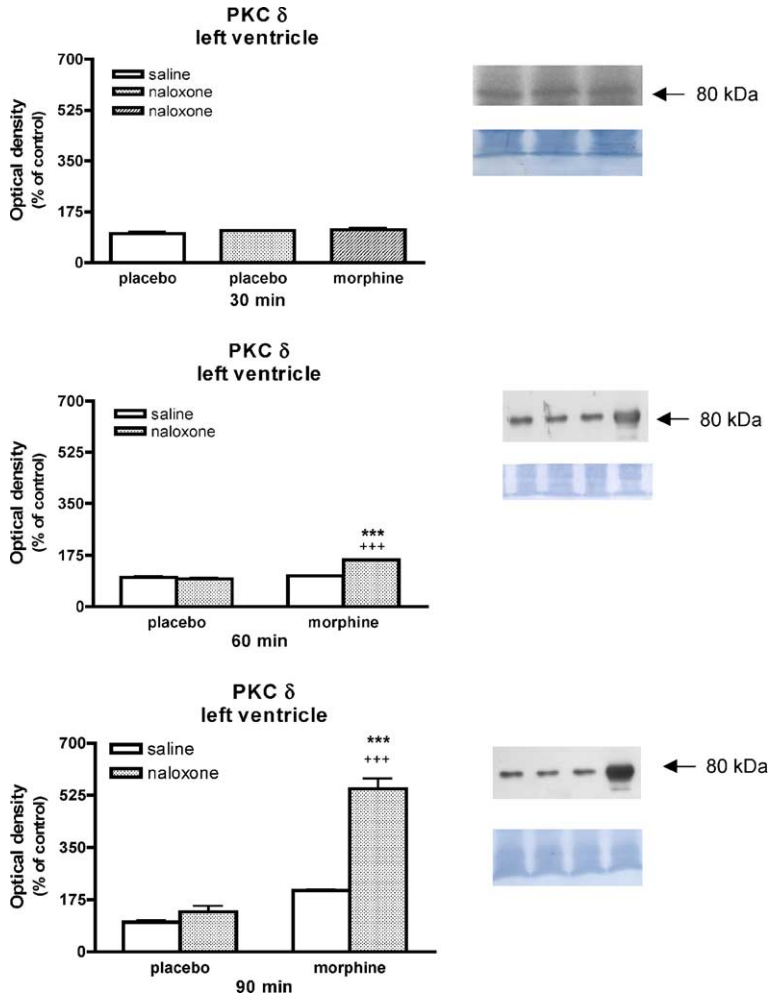

Fig. 6. Western blotting analysis of protein kinase (PKCδ) immunoreactivity levels in the left ventricle in different experimental groups. Animals were decapitated 30 min, 60 min or 90 min after saline or naloxone injection. The immunoreactivity corresponding to PKCδ is expressed as a percentage of that in the control group (placebo+saline; defined as 100% value). Data are means±S.E.M., $n=4$–6. ***$P<0.001$ versus morphine+saline; +++$P<0.001$ versus placebo+naloxone. Right panels: representative band from autoradiograms at the known apparent molecular weight for PKCδ. Similar loading and transfer were ascertained by cutting the lower portion of the blot and staining for total protein with Amido Black.

7 days, the level of PKCδ 30, 60 or 90 min after naloxone injection was similar to that of the control group receiving saline instead of naloxone. As Figs. 7 and 8 depict, rats dependent on morphine and given naloxone also showed significant ($p<0.001$) elevation of PKCζ in the right and left ventricle compared with the control group receiving naloxone or with the dependent group receiving saline.

4. Discussion

In the present investigation, we have quantified the immunoreactivity of PKA-Cα subunit, the major catalytic isoform of PKA in most mammalian tissues (Uhler et al., 1986) and different isoforms of PKC (the conventional PKCα, the novel PKCδ and the atypical PKCζ) during morphine withdrawal.

It is known that chronic morphine alters the levels and/or activity of various mu-opioid receptor signalling elements. These chronic adaptive molecular mechanisms involve some protein kinases which are relevant for signalling processes involving protein phosphorylation (Nestler and Aghajanian, 1997; Liu and Anand, 2001). Many investigators have shown that chronic morphine administration increased the levels of adenylate cyclase and PKA in the central nervous system (Nestler and Aghajanian, 1997; Nestler, 1992; Benavides et al., 2005). Thus, upregulation of PKA after chronic use of morphine has been suggested as one of the molecular mechanisms of opioid tolerance and addiction (Nestler and Aghajanian, 1997). Additional, several recent studies have indicated that PKC is involved in opioid addiction (Williams et al., 2001; Cerezo et al., 2002).

Despite substantial evidence that PKA in the central nervous system are involved in opioid tolerance/dependence, the possible functional disturbances of the PKA in the heart after chronic morphine treatment and upon drug withdrawal are still unknown.

The results of the present study show that, in the right and left ventricle, naloxone-induced morphine withdrawal is associated with increased levels of PKA 30, 60 or 90 min after the opioid antagonist administration. The heart from rats dependent on morphine is characterized by a marked response

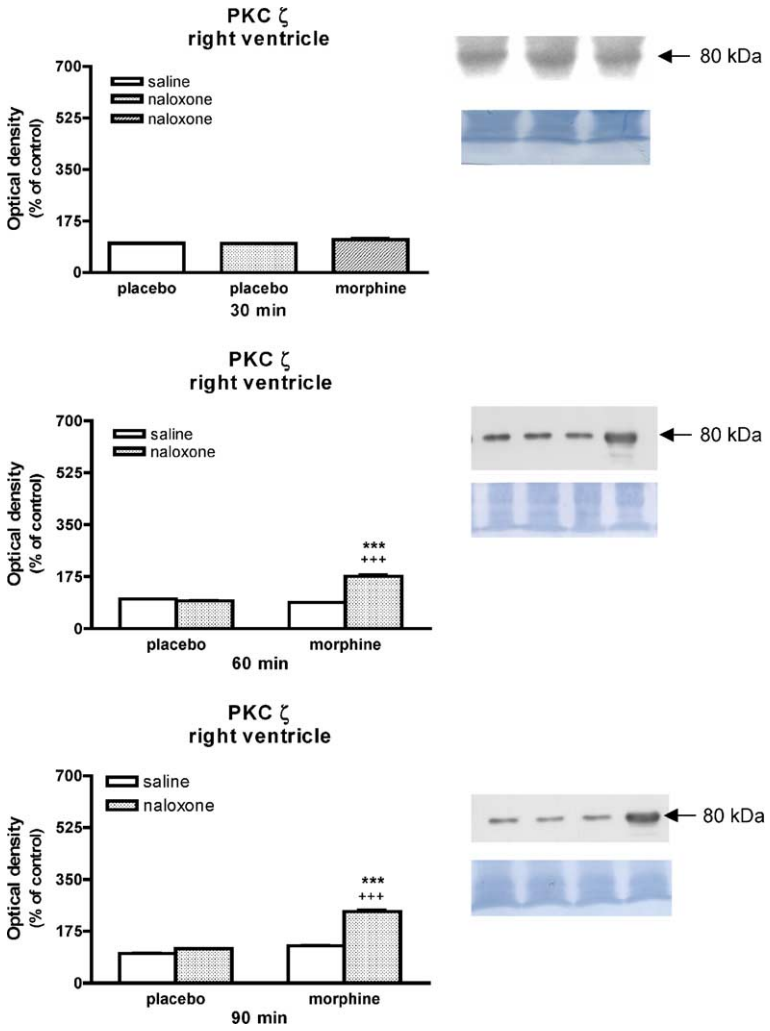

Fig. 7. Western blotting analysis of protein kinase (PKCζ) immunoreactivity levels in the right ventricle in different experimental groups. Animals were decapitated 30 min, 60 min or 90 min after saline or naloxone injection. The immunoreactivity corresponding to PKCζ is expressed as a percentage of that in the control group (placebo+saline; defined as 100% value). Data are means±S.E.M., $n=4$–6. ***$P<0.001$ versus morphine+saline; +++$P<0.001$ versus placebo+naloxone. Right panels: representative band from autoradiograms at the known apparent molecular weight for PKCζ. Similar loading and transfer were ascertained by cutting the lower portion of the blot and staining for total protein with Amido Black.

after naloxone-precipitated withdrawal. Thus, previous studies in our laboratory have demonstrated that withdrawal from morphine is associated with a marked increase in the ventricular levels of cAMP in parallel with an enhancement of noradrenaline turnover (Milanés et al., 2000). The increase of noradrenaline turnover was prevented by α_2-adrenoceptor antagonists (Milanés and Laorden, 2000) and by HA-1004 (a selective PKA inhibitor) (Martínez et al., 2003). Additionally, recent studies from our laboratory showed that morphine withdrawal induced the expression of Fos protein in the heart (González-Cuello et al., 2003). However, the intracellular signal transduction pathway responsible for the onset of the heart activation is still unknown. Present results indicate that morphine withdrawal increases immunoreactivity levels of PKA. This up-regulation of CAMP/PKA signal transduction pathway could represent an early step in the enhancement of noradrenaline turnover and Fos expression observed during morphine withdrawal in the heart (González-Cuello et al., 2003; Martínez et al., 2003).

Repeated administration of morphine leads to molecular adaptations that may contribute to drug dependence, which is characterized by a somatic abstinence syndrome. According to the present results, an up-regulation of the cAMP pathway has been observed during morphine withdrawal (Nestler and Aghajanian, 1997) in specific brain areas, including activation of PKA and phosphorylation of cAMP response element binding protein (CREB) (Blendy and Maldonado, 1998; Williams et al., 2001). Furthermore, precipitated morphine withdrawal has been shown to increase several indices of CREB function, including c-*fos* expression in the rat. In addition, it has been demonstrated that tyrosine hydroxylase is directly phosphorylated by PKA and that phosphorylation regulates tyrosine hydroxylase activity. The enzyme phosphorylated has a higher affinity for the pteridine cofactor, accelerating the synthesis of noradrenaline (Kumer and Vrana, 1996). Thus, it is possible that the up-regulation of PKA observed in the present study might contribute to activation of the heart noradrenergic system and the enhancement of Fos expression that were seen during morphine withdrawal.

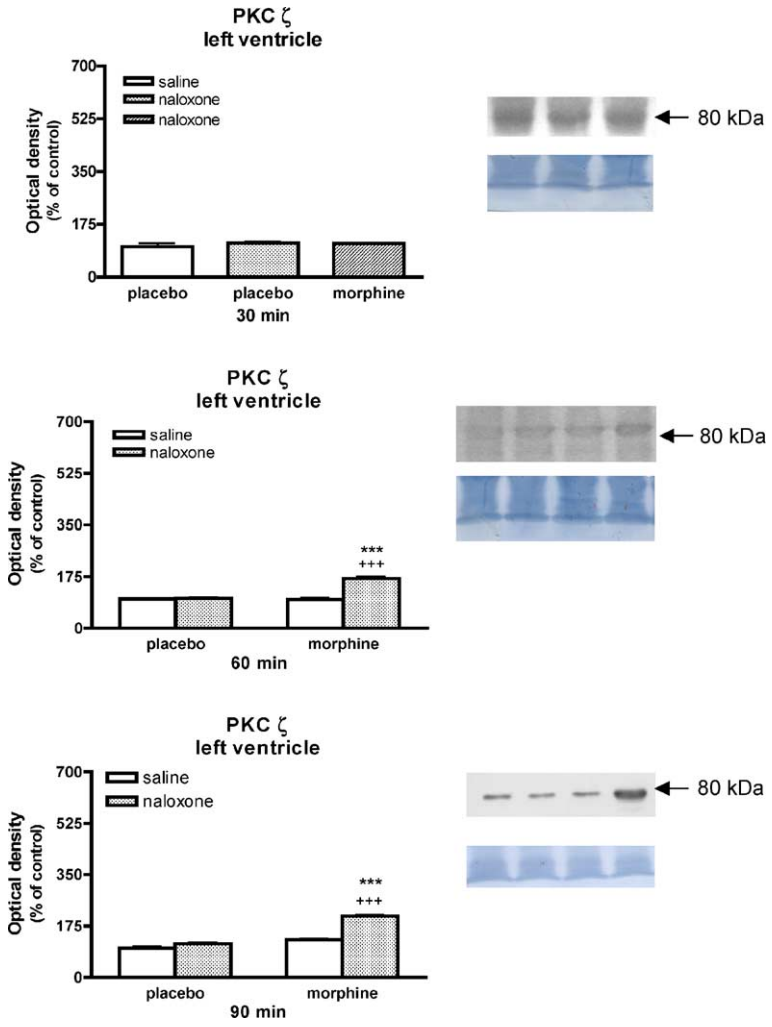

Fig. 8. Western blotting analysis of protein kinase (PKCζ) immunoreactivity levels in the left ventricle in different experimental groups. Animals were decapitated 30 min, 60 min or 90 min after saline or naloxone injection. The immunoreactivity corresponding to PKCζ is expressed as a percentage of that in the control group (placebo+saline; defined as 100% value). Data are means±S.E.M., $n=4$–6. ***$P<0.001$ versus morphine+saline; +++$P<0.001$ versus placebo+naloxone. Right panels: representative band from autoradiograms at the known apparent molecular weight for PKCζ. Similar loading and transfer were ascertained by cutting the lower portion of the blot and staining for total protein with Amido Black.

Despite the discovery of multiple PKC isozymes with distinct distributions and functions, the contribution of specific PKC isozymes during morphine withdrawal has not been investigated. Therefore, we examined the possible changes in the expression of conventional, novel and atypical PKC isoforms after naloxone precipitated withdrawal to asses their contribution to the adaptive changes that occur in the heart during morphine withdrawal. The results of the present study demonstrate that chronic treatment with morphine is associated with no changes in PKCα, whereas naloxone-induced morphine withdrawal results in a marked down-regulation in the immunoreactive levels of PKCα. PKCα isoforms are classical PKC which are Ca^{2+}, phospholipids dependent protein kinases and play crucial role in the signal transduction (Nishizuka, 1988). It is known that PKC isoforms exist in an inactive state within the cytosol but become translocated to the plasma membrane by various stimuli. Activation and translocation of the PKC isoforms to specific subcellular sites is believed to confer distinct physiological actions for each PKC isoform and is thought to be achieved by the binding of each activated PKC isoform to specific anchoring proteins (Way et al., 2000). The translocation of PKC is believed to be its primary mode of activation (Tanaka and Nishizuka, 1994). After the translocation, PKC is known to be rapidly proteolyzed by proteinases (down-regulation), to an inactive isoform (Nishizuka, 1995). The results of the present study show that naloxone-precipitated morphine withdrawal exerts a down-regulation of PKCα isoform. Therefore, it is likely that the effect of morphine withdrawal on PKC immunoreactivity in the heart may be due to an increased activity of PKC in this area.

It has been reported that opioids are capable of activating PKC, as evidenced by enhanced translocation of the enzyme to the cell membrane (Kramer and Simon, 1999). Furthermore, it has been confirmed that PKC was markedly activated in different brain areas in morphine-withdrawn rats (Tokuyama et al., 1995; Busquets et al., 1995). The present findings clearly show that PKCα immunoreactivity in the rat heart is reduced after precipitated opioid withdrawal, which might indicate the

possibility that abstinence could induce the activation of PKCα.

On the other hand, the levels of PKCδ and PKCζ increased after naloxone administration to morphine-dependent rats. In agreement with these results different studies have demonstrated that the Ca^{2+}-dependent or conventional PKCα, but not the Ca^{2+}-independent PKC isoforms, are down-regulated in brains of opiates addicts (Liu, 1996; García-Sevilla et al., 1997). It has been established that PKC down-regulation by several agents requires enzyme translocation and association with the plasma membrane, which is the case for PKCα but not for PKCζ (Liu, 1996). In addition, an up-regulation of PKCγ, but not PKCα, in the limbic forebrain from rats chronically treated with morphine has been demonstrated (Narita et al., 2001). In another study, a continuous morphine infusion resulted in higher PKC activity and higher PKCα and γ levels in the dorsal spinal horn (Granados-Soto et al., 2000). The present findings indicate that the PKCδ and ζ immunoreactivity in the heart are increased after naloxone administration to morphine-dependent rats, which indicate the possibility that abstinence results in the activation of PKC. Since PKC has been reported to be involved in morphine dependence, it is possible that activation of this kinase may be part of the long-term intracellular mechanisms that underlie opioid dependence.

In summary, the results of present study suggest that PKA and PKC are activated after naloxone-induced morphine withdrawal and provide new information on the mechanisms implicated in the cardiac adaptive changes that are seen in the heart after naloxone induced withdrawal.

Acknowledgements

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