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Parallel modulation of receptor for activated C kinase 1 and protein kinase C-α and β isoforms in brains of morphine-treated rats

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1 Receptor for activated C kinase 1 (RACK1) is an intracellular receptor for protein kinase C (PKC) that regulates the cellular enzyme localization. Because opiate drugs modulate the levels of brain PKC (Ventayol *et al*., 1997), the aim of this study was to assess in parallel the effects of morphine on RACK1 and PKC-α and β isozymes densities in rat brain frontal cortex by immunoblot assays.

2 Acute morphine (30 mg kg^{-1}, i.p., 2 h) induced significant increases in the densities of RACK1 (33%), PKC-α (35%) and PKC-β (23%). In contrast, chronic morphine (10–100 mg kg^{-1}, i.p., 5 days) induced a decrease in RACK1 levels (22%), paralleled by decreases in the levels of PKC-α (16%) and PKC-β (16%).

3 Spontaneous (48 h) and naloxone (2 mg kg^{-1}, i.p., 2 h)-precipitated morphine withdrawal after chronic morphine induced marked up-regulations in the levels of RACK1 (38–41%), PKC-α (51–52%) and PKC-β (48–62%).

4 In the same brains and for all combined treatments, there were significant positive correlations between the density of RACK1 and those of PKC-α ($r=0.85$, $n=35$) and PKC-β ($r=0.75$, $n=32$).

5 These data indicate that RACK1 is involved in the short- and long-term effects of morphine and in opiate withdrawal, and that RACK1 modulation by morphine or its withdrawal is parallel to those of PKC-α and β isozymes. Since RACK1 facilitates the PKC substrate accessibility, driving its cellular localization, the coordinate regulation of the PKC/RACK system by morphine could be a relevant molecular mechanism in opiate addiction.

Keywords: Morphine; opiate addiction; opioid receptors; protein kinase C; receptor for activated C kinase 1; rat brain; cytoskeleton

Abbreviations: AKAP, A kinase anchoring proteins; G protein, guanine nucleotide-binding regulatory protein; GRK, G protein-coupled receptor kinase; IOD, integrated optical density; PKA, protein kinase A; PKC, protein kinase C; RACK1, receptor for activated C kinase 1

Introduction

Protein kinase C (PKC) isozymes comprise a family of peripheral membrane proteins that phosphorylate and regulate the activity of some G protein-coupled receptors, such as opioid receptors, and other signalling proteins (Chen & Yu, 1994; Maldonado *et al*., 1995). Opiate drugs have been shown to modulate the levels of classical PKC-α and -β isoforms but not the atypical PKC-ζ isoform in brain (Busquets *et al*., 1995; García-Sevilla *et al*., 1997b; Ventayol *et al*., 1997), which in turn may regulate adenylyl cyclase activity (Zhou *et al*., 1994), a major signalling effector used by opioid receptors and which is up-regulated during opioid dependence (Ammer & Schulz, 1997; Nestler & Aghajanian, 1997). Upon activation, PKC translocates from the cytosol to the plasma membrane (Kraft & Anderson, 1983), but several activated PKC isozymes have also been localized on cytoskeletal structures (Mochly-Rosen *et al*., 1990; Kiley & Jaken, 1990), nuclei (Cambier *et al*., 1987) or other cellular structures (Rotem *et al*., 1990).

Receptors for activated C kinase (RACKs) have been shown to bind PKC, although they are not PKC substrates, and modulate the cellular enzyme localization (Mochly-Rosen *et al*., 1991). Because PKC has a variety of potential substrates, its cellular localization restricts its activity to a number of given targets. RACK1, which has some homology with G protein β-subunits, binds PKC with high affinity and specificity (Ron *et al*., 1994). Conformational changes associated with PKC activation expose its autoregulatory pseudo-RACK and pseudo-substrate sites, allowing its binding to RACK1, which stabilizes the active PKC state (Ron & Mochly-Rosen, 1995). Therefore, the role of RACK proteins and other kinase-anchoring proteins, such as AKAPs [A kinase (PKA) anchoring proteins] would be to ensure the accessibility and preferential phosphorylation of specific substrates at defined cellular compartments (Mochly-Rosen, 1995; Pawson & Scott, 1997). In this context, the increase in PKC activity in rat kidney during proximal-tubule regeneration after ischaemia has been shown to be associated with an increased RACK1 expression (Padanilam & Hammerman, 1997). Furthermore, the decrease in PKC activity in brain of aged rats was show to be due to a decrease in RACK1 levels, rather than to changes in the PKC abundance (Pascale *et al*., 1996). All these studies suggest that this intracellular receptor, RACK1, is crucial for PKC localization and activity.

Because opiates modulate the abundance, expression and activity of PKC (Mangoura & Dawson, 1993; Ventayol *et al*., 1997), which may depend on the levels of its intracellular receptor, RACK1 regulation by opiates appears as a relevant

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target for a better understanding of opioid addiction, whose molecular bases remain largely unknown (Nestler & Aghajanian, 1997). In this context, the aim of this study was to assess the acute and chronic effects of morphine, as well as of morphine withdrawal in morphine-dependent rats, on the levels of brain RACK1, and its relation with PKC-α and -β isozymes. A preliminary report of a portion of this study was presented at a meeting of the British Pharmacological Society (Escribá & García-Sevilla, 1998).

Methods

Animals and treatments

Male Sprague-Dawley rats (weighing 250–300 g) were used. The animals were housed under controlled environmental conditions (22°C, 70% humidity, and a 12 h light/dark cycle) with free access to food and water. For the acute treatment, the rats received a single intraperitoneal (i.p.) injection of either 0.9% NaCl vehicle or morphine (30 mg kg^{-1}) and were killed by decapitation 2 h later. For the chronic treatment, the rats were injected i.p. three times daily (at 08 00, 14 00, and 20 00 h) during 5 days with increasing doses of morphine (10–100 mg kg^{-1}) (see Escribá *et al*., 1994, for further details). After this chronic treatment, naloxone (2 mg kg^{-1}, i.p., 2 h)-precipitated withdrawal or spontaneous morphine withdrawal (2–48 h) was induced, which resulted in rapid or delayed withdrawal reactions (data not shown). In the chronic and withdrawal studies, the rats were killed 2 h after the last injection, except for the spontaneous opiate withdrawal group, in which they were killed 2, 12 and 48 h after the last morphine injection. The brain was rapidly removed, and specimens of the cerebral frontal cortex were dissected and stored at −80°C, for no longer than 2 weeks, until use.

Immunoblot analysis and quantitation of RACK1, PKC isozymes and α-tubulin

One-hundred to 150 mg of rat cerebral frontal cortex was homogenized (1 : 15, w v^{-1}) in 20 mM Tris-HCl buffer, pH 7.5, containing sucrose 250 mM, EDTA 5 mM, iodoacetamide 5 mM, phenylmethylsulphonyl fluoride 1 mM, 10 μg ml^{-1} of trypsin/chymotrypsin inhibitor, 10 μg ml^{-1} of leupeptin and 1 μg ml^{-1} of aprotinin. A 200-μl aliquot of the previous mixture was combined with 40 μl of solubilization buffer [160 mM Tris-HCl, pH 6.8, 8% sodium dodecyl sulphate (SDS)] and incubated for 10 min at room temperature. Then, 27 μl of loading buffer [Tris-HCl (pH 6.8) 120 mM, 50% glycerol, 4% SDS, NEM 10 mM, β-mercaptoethanol 58 mM] was added to the samples, which were subsequently boiled for 3 min. Next, 5 to 35 μl of the resulting suspension was submitted to polyacrylamide gel electrophoresis (SDS–PAGE) on 15-well (6 × 8-cm minigels, 1.5 mm thickness) 12% polyacrylamide gels. Proteins were immediately transferred onto nitrocellulose membranes (immunoblotting), that were incubated in phosphate-buffered saline [in mM: NaCl 137, KCl 2.7, Na_2HPO_4 12, and KH_2PO_4 (pH 7.4) 1.38], containing 5% nonfat dry milk, 0.5% bovine serum albumin and 0.1% Tween 20 (blocking solution) for 1 h at room temperature with gentle rocking. Then, the membranes were incubated overnight at 4°C in blocking solution containing the primary antibody at 1 : 2000 dilution (monoclonal anti-RACK1), at 1 : 1000 dilution (polyclonal anti-PKC-α or anti-PKC-β) or at 1 : 10,000 dilution (monoclonal anti-α-tubulin). The secondary antibody, horseradish peroxidase-linked sheep anti-mouse IgG (RACK1 and α-tubulin), or donkey anti-rabbit IgG (PKC isozymes), was incubated at 1 : 5000 dilution in blocking solution at room temperature for 2 h. Immunoreactivity was detected with an enhanced chemiluminescence (ECL) Western blot detection system, followed by exposure to autoradiography film for 1–10 min. Films were scanned with a resolution of 31 μm, and the whole-band integrated optical density (IOD) values were obtained using the 'SigmaGel' (Jandel Scientific, San Rafael, CA, U.S.A.) image analysis software. For the quantitation of these proteins, quadruplicate problem samples, containing ~30 μg of total protein, were evaluated using standard curves (i.e., total protein loaded versus IOD) consisting of five points of different protein content (usually 10–60 μg of protein, resulting in a linear relation) of naive rat brain samples, all loaded on the same gel (Figure 1). This quantitation procedure was repeated at least twice in different gels and experiments, for a minimum of eight measurements for each rat brain sample. Finally, per cent changes with respect to control samples (100%) were calculated. For further details see Escribá *et al*. (1994; 1995) and Ventayol *et al*. (1997). The ECL detection system allowed blots to be stripped and reprobed with the various antibodies in the same brain sample of a given rat (this approach provided the best conditions for correlation analysis between RACK1 and PKC isozymes).

Statistics

Results are expressed as mean ± s.e.mean values. One-way ANOVA, followed by Fisher's multiple comparison test, was used for the statistical evaluations. Pearson correlation coefficients were calculated to test for possible association between variables. The level of significance was chosen as $P = 0.05$.

Materials and drugs

Anti-RACK1 monoclonal antibody (R20620) was purchased from Transduction Laboratories (Lexington, KY, U.S.A.). Anti-PKC antisera were purchased from Boehringer Mannheim (Barcelona, Spain). Anti-α-tubulin monoclonal antibody (Clone DM 1A) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Horseradish peroxidase-linked secondary antibodies (sheep anti-mouse and donkey anti-rabbit IgGs), ECL reagents and autoradiography film (Hyperfilm ECL) were purchased from Amersham (Buckinghamshire, U.K.). Morphine hydrochloride was from Unión Químico-Farmaceútica SAE (Madrid, Spain) and naloxone hydrochloride was from Endo Laboratories Inc. (Garden City, NY, U.S.A). All other chemicals were from Sigma.

Results

Immunoblot analysis, using a specific anti-RACK1 antibody, showed the presence of a unique immunoreactive peptide of ~36-kDa, in the rat brain cortex (Figure 1). A linear relation between the amount of protein loaded on the gel, and the IOD values of the bands was also observed (Figure 1). Moreover, the range of changes for RACK and PKC-α and -β levels, after morphine treatments, led to marked changes in IOD values (e.g., Figure 2). These facts indicated that immunoblotting, followed by image analysis, is an appropriate and reliable method for the detection and quantitation of RACK1 and PKC isozymes, as assessed for other regulatory proteins (Escribá *et al*., 1994; Ventayol *et al*., 1997).

Effects of morphine treatments on RACK1 and PKC-α and -β levels in brain

Acute morphine treatment (30 mg kg^{-1}, i.p., 2 h), compared with saline solution administration, induced a significant increase in the levels of RACK1 (33±5%) in the frontal cortex (Figure 2). This morphine treatment also caused a parallel up-regulation in the levels of PKC-α (35±7%) and PKC-β (23±4%) in the same brain region (Table 1).

In contrast, chronic morphine treatment (10–100 mg kg^{-1}, i.p., 5 days) induced a modest but significant decrease in RACK1 levels (22±3%) in the frontal cortex (Figure 2). Similarly, this chronic morphine treatment also induced significant parallel reductions in the levels of PKC-α (16±5%) and PKC-β (16±5%) in the same brain region (Table 1).

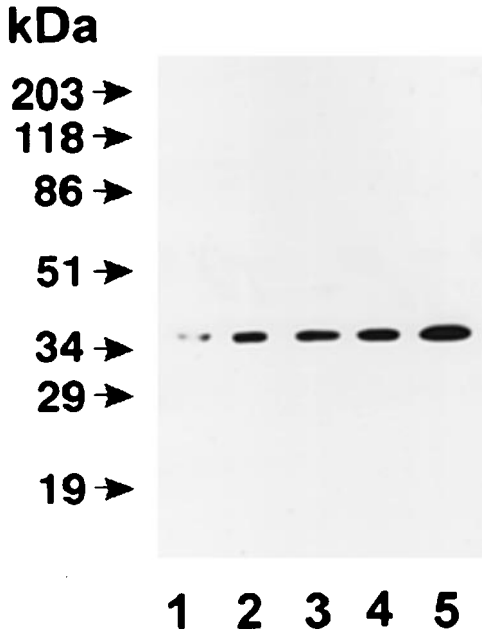

Figure 1 RACK1 immunoreactivity. Representative immunoblotting assay of RACK1 in the rat brain (cerebral cortex), using a specific monoclonal anti-RACK1 antibody. Under the reported conditions, a unique peptide of ~36 kDa was detected. The electrophoretical mobility of this protein was identical to that of a control provided by the antibody manufacturer (data not shown). This figure also shows the concentration-dependence of the signals obtained by immunoblotting, corresponding to a standard curve. Total protein loaded on the gel and IOD values for these bands were: 12 μg and 7.32 IOD units (lane 1); 24 μg and 17.80 IOD units (lane 2); 30 μg and 27.37 IOD units (lane 3); 36 μg and 36.08 IOD units (lane 4) and 48 μg and 53.59 IOD units (lane 5), respectively. The amount of total protein loaded for problem samples was around 30 μg.

Effects of naloxone-precipitated and spontaneous morphine withdrawal on RACK1 and PKC isozymes levels in brain

In morphine-dependent rats, naloxone (2 mg kg^{-1}, i.p.)-precipitated withdrawal induced a rapid (2 h) up-regulation in the levels of RACK1 (19±4%) above saline controls in the frontal cortex (Figure 2). This up-regulation of RACK1

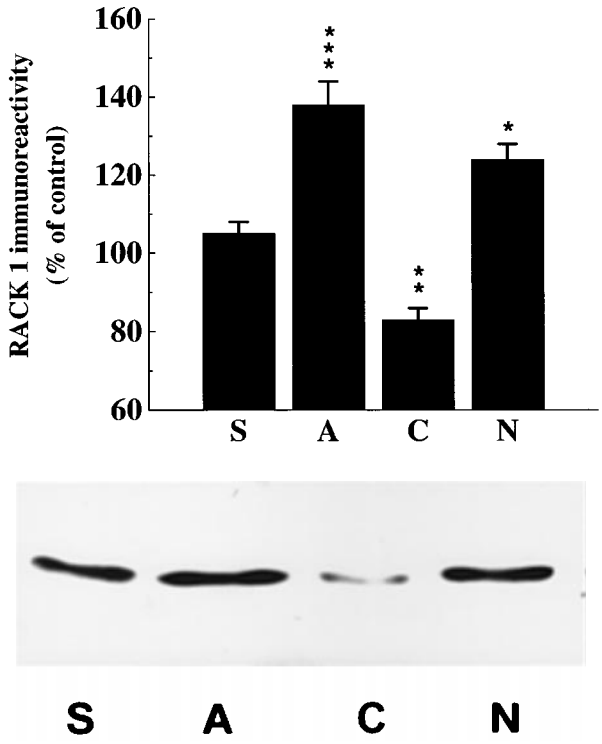

Figure 2 Effects of acute and chronic morphine treatment and naloxone-precipitated withdrawal on RACK1 immunoreactivity in the rat cerebral cortex. Rats were treated i.p. with saline vehicle (S, $n=10$), acute morphine (A, $n=8$; 30 mg kg^{-1}, 2 h), chronic morphine (C, $n=8$; 10–100 mg kg^{-1}, 5 days) or chronic morphine plus naloxone (N, $n=6$; 2 mg kg^{-1}, 2 h). Upper panel: data are mean±s.e.mean (bars) percentages of immunoreactivities in naive rats (untreated group). One-way ANOVA [$F(3,28)=37.15$, $P<0.0001$] followed by a multiple comparison test detected significant increases in RACK1 immunoreactivity after acute morphine and naloxone-precipitated withdrawal in morphine-dependent rats and a significant decrease after chronic morphine treatment: *$P<0.05$; **$P<0.01$; ***$P<0.001$, when compared with saline (S). Lower panel: representative immunoblot for the effects of acute morphine (A), chronic morphine (C) and chronic morphine plus naloxone (N) on RACK1 levels. Total protein loaded on the gel and IOD values were: 32.6 μg and 32.97 IOD units (S), 33.1 μg and 48.2 IOD units (A), 27.4 μg and 9.86 IOD units (C) and 30.3 μg and 37.1 IOD units (N).

Table 1 Effects of morphine and naloxone-precipitated withdrawal on PKC isozymes and α-tubulin immunoreactivity in the rat cerebral cortex

	PKC-α (% of control)	n	*PKC-β* (% of control)	n	*α-tubulin* (% of control)	n
Saline	100±4	8	101±3	6	102±5	6
Acute	135±7†	4	124±7*	8	103±6	4
Chronic	84±5*	7	85±5*	5	102±5	5
Naloxone	135±4†	4	147±7†	3	97±4	3

Rats were treated i.p. with saline vehicle, acute morphine (30 mg kg^{-1}, 2 h), chronic morphine (10–100 mg kg^{-1}, 5 days), or chronic morphine plus naloxone (2 mg kg^{-1}, 2 h). Data are mean±s.e.mean percentages of immunoreactivity in naive rats (untreated group). n is the number of animals per group. One way ANOVA [$F(3,19)=23.91$, $P<0.0001$] followed by a multiple comparison test detected significant changes in the levels of brain PKC isozymes after the various treatments. *$P<0.05$; †$P<0.005$.

represented a rebound of 41% when compared with the protein levels after (2 h) chronic morphine administration (Figure 2). Naloxone-precipitated withdrawal also induced marked increases in the abundance of PKC-α ($35\pm4\%$) and PKC-β ($46\pm7\%$), which represented rebounds of 52 and 62%, respectively, when compared with enzyme levels after (2 h) chronic morphine treatment (Table 1). Similarly, spontaneous opiate withdrawal (48 h) also resulted in up-regulation of RACK1 levels ($18\pm4\%$) in the frontal cortex, although the time-course was slower than that associated with naloxone-precipitated withdrawal (Figure 3). The levels of PKC-α and PKC-β in brain during spontaneous morphine withdrawal exhibited a very similar pattern of up-regulation (34 ± 7 and $32\pm6\%$ at 48 h, respectively) to that of their intracellular receptor, RACK1 (Figure 3). This up-regulation of RACK1, PKC-α and PKC-β represented rebounds of 38, 52 and 47%, respectively, when compared with the protein levels after (2 h) chronic morphine treatment.

The acute and chronic treatments with morphine and the naloxone-precipitated morphine withdrawal did not modify significantly the immunoreactivity of α-tubulin, a cytoskeletal protein used as loading control (Table 1), which discounted possible nonspecific effects of the opiate on the modulation of RACK1 and PKC abundance.

Correlations between the densities of RACK1 and PKC isozymes in brain

Because of the parallel modulation of RACK1 and PKC-α and β isozymes after the various morphine treatments and withdrawal states, the possible existence of an *in vivo* relation between the ligand (PKC) and the intracellular receptor (RACK1) was assessed through correlation analyses. In the same cerebral cortices, there were significant positive correlations between the density of RACK1 and the densities of PKC-α ($r=0.85$, $n=35$, $P<0.001$) and PKC-β ($r=0.75$, $n=32$, $P<0.001$) (Figure 4). These strong correlations clearly indicated that morphine (and possibly other μ-opioid receptor agonists) modulate brain PKC and RACK1 levels through a coordinate mechanism.

Discussion

Morphine and related opiates act primarily at the μ-opioid receptor, and the understanding of the molecular consequences of morphine on signalling elements modulated by this receptor is of relevance in opiate addiction (Nestler & Aghajanian, 1997). μ-Opioid receptors are G protein-coupled receptors at which endomorphin-1 and -2 appear to be endogenous and selective agonists (Zadina *et al.*, 1997). They are involved in nociceptive processing and morphine-induced analgesia, as well as in the development and expression of morphine

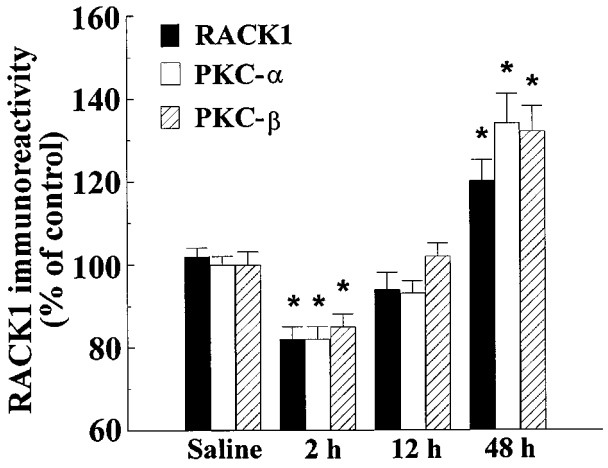

Figure 3 Effects of chronic morphine treatment and spontaneous withdrawal on RACK1, PKC-α and PKC-β immunoreactivities in the rat cerebral cortex. Rats were treated i.p. with morphine (10–100 mg kg^{-1}, 5 days) and killed 2 h ($n=3$), 12 h ($n=3$) or 48 h ($n=3$) after the last morphine injection. Data are mean$\pm$s.e.mean (bars) percentages of immunoreactivity of saline-treated rats ($n=3$). One-way ANOVA [F(3, 8)=26.3–29.3, $P<0.001$] followed by a multiple comparison test detected significant changes in RACK1 and PCK isozymes immunoreactivities during withdrawal: $^*P<0.05$, when compared with saline.

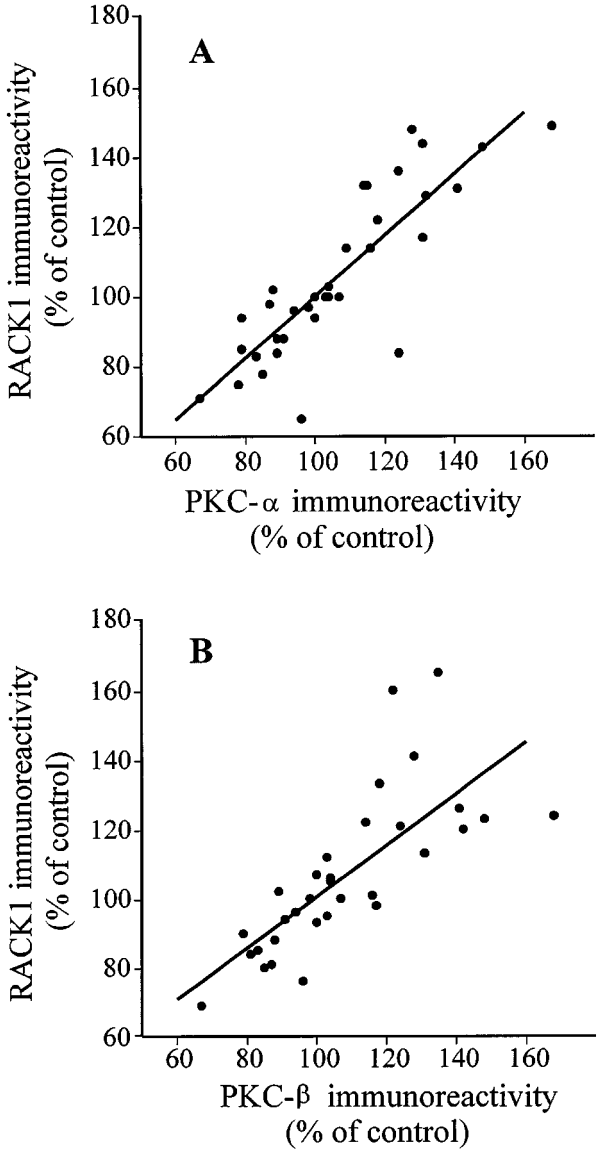

Figure 4 Scatterlots and linear regression lines showing the relation between RACK1 and PKC-α (A) or PKC-β (B) in cerebral cortices of the same rats after various treatments (saline vehicle, acute and chronic morphine, chronic morphine plus naloxone and spontaneous morphine withdrawal). Values are expressed as percentage immunoreactivity in naive rats. See Figures 2 and 3 for further details. Data were best described by the expressions: PKC-α, $y=12+0.88x$ ($r=0.85$, $n=35$, $P<0.001$) and PKC-β, $y=26+0.77x$ ($r=0.75$, $n=32$, $P<0.001$).

addiction (Matthes *et al*., 1996; Sora *et al*., 1997). In this context, it is known that morphine and other opiate drugs alter the levels and/or activity of various μ-opioid receptor signalling elements, such as G proteins (Escribá *et al*., 1994; Manji *et al*., 1997), adenylyl cyclase and PKA (Nestler & Tallman, 1988; Zhou *et al*., 1994), G protein-coupled receptor kinase 2 (GRK2) (Terwilliger *et al*., 1994; Ozaita *et al*., 1998), and PKC-α and -β isoforms (Busquets *et al*., 1995; Ventayol *et al*., 1997). These chronic adaptive molecular mechanisms in opiate addition involve some protein kinases (e.g., PKC, PKA and GRK), which are relevant for a wide variety of cellular regulatory and signalling processes involving protein phosphorylation (Nestler & Aghajanian, 1997). Furthermore, translocation and compartmentalization of these peripheral proteins are crucial for their activities, and such processes are regulated by protein-protein and protein-lipid interactions (Mochly-Rosen *et al*., 1991; Slater *et al*., 1994; Mochly-Rosen, 1995; Escribá *et al*., 1995; 1997). Thus, RACK1 binds activated PKC, through WD40 repeats found along its amino acid sequence, which interact with C2 (PKC-α, -β and -γ) or V1-3 (PKC-ζ, -δ and -ε) regions of the kinase (Ron *et al*., 1994; 1995; Ron & Mochly-Rosen, 1995; Yedovitzky *et al*., 1997). PKC contains a pseudo-RACK site that avoids its binding to RACK proteins before activation, similar to the pseudo-substrate site that stabilizes the inactive state of the enzyme (Ron & Mochly-Rosen, 1995).

The present work was designed to seek for possible effects of morphine on the abundance of brain RACK1, and to assess the relation with the levels of some of its ligands (PKC-α and -β isozymes), after various morphine treatments. Acute (33% increase) and chronic (22% decrease), morphine treatments induced opposite modulations on brain RACK1 levels. In morphine-dependent rats, naloxone-precipitated withdrawal resulted in a rapid and marked RACK1 up-regulation (41% increase). Similarly, spontaneous opiate withdrawal induced a slower but progressive increase in RACK1 levels (38% increase, at 48 h). In a similar fashion, all the above treatments were associated with parallel changes in the levels of PKC-α and -β isozymes in brain. It is feasible that the rapid changes in RACK1/PKC levels after acute morphine or naloxone-precipitated withdrawal were related to the overexpression of some immediate early genes (c-*fos* and *junB*), which have been shown to be rapidly induced by opiates (Hayward *et al*., 1990; Liu *et al*., 1994).

On the other hand, despite the amino acid homology found for RACK1 and the G protein β-subunit (Ron *et al*., 1994) their regulation by opiate drugs appear to be different. Thus, chronic morphine or heroin treatment induced a marked increase in the levels of Gβ, whereas acute morphine or heroin and naloxone-precipitated withdrawal in opiate-dependent rats did not significantly change its density in the brain (Escribá *et al*., 1994; Ventayol *et al*., 1997). The remarkable parallelism between the levels of RACK1 and those of PKC-α and -β after morphine treatment or withdrawal has not been found for other proteins involved in opioid signal transduction. Thus, G protein α- and β-subunits (Escribá *et al*., 1994; Ventayol *et al*., 1997), GRK2 (Terwilliger *et al*., 1994; Ozaita *et al*., 1998) adenylyl cyclase and PKA (Nestler & Tallman, 1988; Rasmussen *et al*., 1990), μ-opioid receptors and PKC-ζ (García-Sevilla *et al*., 1997b) levels show different modulations to those of RACK1 and PKC-α and -β after one or more of the above treatments (acute morphine, chronic morphine, spontaneous and naloxone-precipitated withdrawal). These facts may suggest common opiate-induced regulatory mechanisms for RACK1 and PKC, and distinct regulatory processes for other signalling proteins.

In contrast, the patterns of regulation of RACK1, PKC-α and -β isozymes were very similar for all the treatments tested in the current study. In fact, there were significant positive correlations between the levels of RACK1 and those of both PKC isozymes when measured in the same brains, suggesting that the functional relation between RACK1 and PKC is a relevant system common to processes that alter the activity of this kinase. These results also suggest that the modulation of RACK1 by morphine (and possibly by neurotransmitters, hormones and drugs that regulate PKC activity) is related to PKC regulation, but not to that of the structurally related Gβ protein.

The down-regulation of the 68-kDa neurofilament (NF-L) protein in brains of morphine-dependent rats (Beitner-Johnson *et al*., 1992) and human opiate addicts (García-Sevilla *et al*., 1997a), as well as the up-regulation of the glial fibrillary acidic protein (GFAP) in chronic-morphine treated rats (Beitner-Johnson *et al*., 1993), indicate that certain intermediate filament proteins also play important roles in the adaptive molecular mechanisms related to opiate addiction. In fact, RACKs have been associated with the differential localization of activated PKC isozymes: PKC-α and -ε translocate to the cellular periphery, while PKC-δ and -ζ translocate to perinuclear sites in pancreatic β-cells (Yedovitzky *et al*., 1997). However, this active role in cellular communication does not appear to be shared by other cytoskeletal proteins: β-tubulin, actin, tropomyosin, tau phosphoprotein, vimentin and synapsin levels remained unchanged in the rat brain after chronic morphine treatment (Beitner-Johnson *et al*., 1992).

In conclusion, the results of the current study indicate that (i) the regulation of RACK1 density in the brain is an adaptive molecular mechanism used by μ-opioid (and possibly other) receptors; (ii) there is a parallel modulation of brain RACK1 and its ligands PKC-α and -β after the various morphine treatments and withdrawal states; (iii) some of the alterations in signal transduction associated with opiate addiction could be linked to alterations in RACK1 levels and (iv) RACK1 might be involved in the molecular and cellular mechanisms underlying opiate tolerance, dependence and withdrawal.

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