Sigma-1 receptors amplify dopamine D1 receptor signaling at presynaptic sites in the prelimbic cortex

Yingmei Fu, Yanyan Zhao [1], Wenjie Luan, Lian-Yian Dong, Yi Dong, Bin Lai, Yanhua Zhu, Ping Zheng *

State Key Laboratory of Medical Neurobiology, Shanghai Medical College and Institutes of Brain Science, Fudan University, Shanghai, China

ABSTRACT

Sigma-1 receptors are highly expressed in the brain. The downstream signaling mechanisms associated with the sigma-1 receptor activation have been shown to involve the activation of protein kinase C (PKC), the control of Ca^2 homoeostasis and the regulation of voltage- and ligand-gated ion channels. But few studies examined the regulatory effect of sigma-1 receptors on metabotropic receptor signaling. The present paper studied the regulatory effect of sigma-1 receptors on the signaling of dopamine D1 receptors, one of metabotropic receptors, by examining the effect of sigma-1 receptor agonists on the D1 receptor agonist-induced cAMP-dependent protein kinase (PKA) activation at presynaptic sites using the synaptosomes from the prelimbic cortex. The results showed that sigma-1 receptor agonists alone had no effects on the PKA activity, but could amplify the D1 receptor agonist-induced PKA activation. The sigma-1 receptor agonist also amplified the membrane-permeable analog of cAMP- and the adenylyl cyclase (AC) activator-induced PKA activation, but did not on the D1 receptor agonist-induced AC activation. The conventional PKC (cPKC), especially the PKCβI, and the extracellular Ca^{2+} influx through L-type Ca^{2+} channels might play key roles in the amplifying effect of the sigma-1 receptor agonists. The activation of PKC by sigma-1 receptor agonists was the upstream event of the increase in the intrasynaptosomal Ca^{2+} concentration. These results suggest that sigma-1 receptors may amplify the D1 receptor agonist-induced PKA activation by sigma-1 receptors - cPKC (especially the PKCβI) - L-type Ca^{2+} channels - Ca^{2+} - AC and/or cAMP signaling pathway.

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1. Introduction

Sigma receptors, recognized as unique receptors, are widely distributed in the mammalian brain. Sigma receptors have been known to be implicated in numerous physiological and pathophysiological processes such as learning and memory, schizophrenia, drug-seeking behaviors and psychostimulant-induced behavioral sensitization [1]. However, many important questions involving the downstream signaling pathways of sigma receptors remain to be studied.

Sigma receptors contain two subtypes, sigma-1 and sigma-2 receptors. The sigma-1 receptor has been cloned and its predicted amino acid sequence suggests two transmembrane domains, demonstrating that it does not correspond to a traditional G-protein-coupled receptor [2]. One of the earliest questions about the downstream signaling mechanisms associated with sigma-1 receptor activation involved their possible coupling to G-proteins [3]. This issue has been studied with different experimental approaches, but the results are contradictory. Some sigma-1 agonists seemed to act through G-proteins, whereas others did not [4,5]. Although the coupling of sigma-1 receptors to G-proteins remains controversial, the downstream signaling mechanisms of the sigma-1 receptors have been shown to involve the activation of protein kinase C (PKC), the control of Ca^2 homoeostasis and the regulation of voltage- and ligand-gated ion channels such as Ca^{2+} channels, transient outward K^+ channels, Ca^{2+}-dependent K^+ channels and NMDA-gated ion channels [6–8]. In addition, studies by Hayashi and Su et al showed that the sigma-1 receptors could exist on lipid rafts at the endoplasmic reticulum (ER) membrane and modulate intracellular Ca^{2+} mobilization at the ER when translocated from lipid droplets on the ER after stimulated by agonists [3,9].

In our previous study, when we examined the mechanism of the effect of neurosteroid dehydroepiandrosterone sulphate (DHEAS) on presynaptic glutamate release in the prelimbic cortex, we found that the sigma-1 receptor antagonist partially blocked the effect of DHEAS, but the dopamine D1 receptor antagonist completely blocked the effect of DHEAS [10]. This result suggests that it is through D1 receptors that sigma-1 receptors produce the promoting effect on glutamate release because D1 receptor antagonist can completely block this effect. In addition, some behavioral evidence suggested that sigma-1 receptors might induce behavioral changes via their actions on D1 receptor [11]. However, the direct evidence supporting that

* Corresponding author. State Key Laboratory of Medical Neurobiology, Fudan University Shanghai Medical College, 138 Yixueyuan Road, Shanghai, 200032, People's Republic of China. Tel.: +86 21 54237437; fax: +86 21 64174579.
   E-mail address: pzheng@shmu.edu.cn (P. Zheng).
   [1] Co-first author.

sigma-1 receptors can amplify D1 receptor signaling is still lacking. In the present paper, we studied the regulatory effect of sigma-1 receptors on the signaling of dopamine D1 receptors by examining the effect of sigma-1 receptor agonists on the D1 receptor agonist-induced cAMP-dependent protein kinase (PKA) activation at presynaptic sites using the synaptosomes from the prelimbic cortex. In addition, since considerable morphological and functional evidence have shown that synaptosomes are sealed particles that contain vesicles, viable mitochondria and all the components necessary to store, release and retain neurotransmitters [12], we also further investigate the mechanism underlying the amplifying effect of sigma-1 receptor agonists on the D1 receptor agonist-induced PKA activation using pharmacological approaches combined with the measure of the intrasynaptosomal Ca^{2+} concentration.

2. Materials and methods

2.1. Synaptosome preparation

Male Sprague-Dawley rats (200-240 g) were anesthetized with chloral hydrate (400 mg/kg, i.p.). All experimental procedures conformed to Fudan University as well as international guidelines on the ethical use of animals and all efforts were made to minimize the number of animals used and their suffering. Synaptosomes were prepared as described previously [13]. The prelimbic cortex was dissected and homogenized in 0.32 M sucrose solution at 4 °C using the Art-Miccra D-8 tissue grinder with a motor-driven pestle rotating at 900 rpm. The homogenate was centrifuged at $3000 \times g$ for 3 min at 4 °C. The supernatant (S1) was centrifuged at $14500 \times g$ for 12 min at 4 °C. The pellet (P2) was resuspended and loaded onto Percoll gradients consisting three steps of 23, 10 and 3% Percoll in 0.32 M sucrose additionally containing 1 mM EDTA and 250 μM DTT. The gradients were centrifuged at $32500 \times g$ for 6.5 min at 4 °C. Synaptosomes were harvested from the interface between the 23 and 10% Percoll layers and washed in Hanks' balanced salt solution (HBSS) containing 140 mM NaCl, 5 mM KCl, 5 mM $NaHCO_3$, 1 mM $MgCl_2$, 1.2 mM Na_2HPO_4, 10 mM glucose and 20 mM HEPES, pH 7.4. Washed synaptosomes were centrifuged at $27000 \times g$ for 15 min at 4 °C. The protein concentration of the synaptosomes was determined by the method of Lowry et.al [14] with the bovine serum albumin as the standard protein.

2.2. Assessment of PKA activity

The activity of PKA was assessed with the PepTag Non-Radioactive PKA Assay Kits from Promega. The assay was based on the change in the net charge of the fluorescent PKA substrate before and after phosphorylation. This change in the net charge of the substrate allowed the phosphorylated and nonphosphorylated version of the substrate to be rapidly separated on an agarose gel at neutral pH. The PKA activity was quantified based on the intensity of the fluorescence of the phosphorylated peptide and no internal standard was used [15].

2.3. Assessment of AC activity

The assessment of AC activity was performed using cAMP-Glo Assay (Promega) following the protocol of the manufacturer [16]. Briefly, the synaptosomes in each well of the assay plate were treated with 1X phosphate-buffered saline (PBS) containing phosphodiesterase inhibitors IBMX and Ro20-1724 to prevent the degradation of cAMP. Accumulation of cAMP is used as an index of the AC activity. The cAMP is detected using the detection solution provided in the kit after cell lysis. The luminescence was measured using a MDS SpectraMax L (Molecular Devices Corporation). A standard curve was performed in parallel for each experiment using serial dilutions of cAMP provided with the kit.

2.4. Western blotting

Immunoblot analysis of sigma-1 receptors was performed on synaptosomes. The synaptosomal pellets were dissolved with $2 \times$ Laemmli sample buffer (100 mM Tris-HCl, 20% glycerol, 4% SDS, pH 6.8) at 4 °C for 10 min [17]. The lysate was centrifuged at 12,000 rpm for 10 min at 4 °C [18]. Protein concentrations were determined using a BCA kit (Pierce Chemicals, Rockford, IL). The samples were treated with the SDS sample buffer at 95 °C for 5 min, loaded on a 10% SDS polyacrylamide gel and blotted to a PVDF membrane. Each blot was incubated with a normal rabbit IgG (1:200, Invitrogen) or a rabbit anti-sigma-1 receptor antibody (1:200, Invitrogen; 1:500, from Hayashi and Su) or a monoclonal anti-α-tubulin antibody (1:4000, Santa Cruz Biotechnology). The horseradish peroxidase development system as previously described [19] was used for immunodetection. The immunoreactivity of the sigma-1 receptors was normalized to that of α-tubulin. Each experiment was repeated at least five times.

Immunoblot analysis of cPKCβI was performed on cytosolic and membrane fractions of synaptosomes prepared according to methods described previously [20,21]. Briefly, synaptosomal pellets were transferred immediately to ice-cold homogenization buffer (20 mM Tris-HCl, pH 7.5, 2 mM EDTA, 2 mM EGTA, 2 mM DTT, 50 μg/ml aprotinin, 48 μg/ml leupeptin, 5 μM pepstatin A, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM sodium vanadate, and 50 mM NaF) and then lysed by sonication and centrifuged at $100,000 \times g$ for 1 h. The supernatant was designated to the cytosolic fraction. The pellets were incubated on ice for 10 min in homogenization buffer containing 0.3% Triton X-100 followed by centrifugation at $100,000 \times g$ for 30 min to obtain the supernatant called the membrane fraction. Protein concentrations were determined using a Bradford assay kit (Bio-Rad Laboratories; Hercules, USA). Equal amounts (30 μg) of protein from different experimental groups were loaded on a 8% SDS polyacrylamide gel, and blotted to a PVDF membrane, incubated with Rabbit polyclonal cPKCβI antibody (1:1000; Abcam, Cambridge, UK). In order to normalize for protein loading, we used a β-actin antibody (1:4000; Santa Cruz Biotechnology, Inc., Santa Cruz, Bergheimer, Germany). The proportion of membrane-bound cPKCβI was expressed as the amount in membranes ($A_{membrane}$) relative to the total cellular amount, according to $A_{membrane}/(A_{membrane} + A_{cytosol})$.

2.5. Measurement of the intrasynaptosomal Ca^{2+} concentration

Purified synaptosomes were loaded with the acetoxymethyl ester derivative of the calcium-sensitive fluorescent dye Fluo-3 AM (5.0 μM) for 30 min at 37 °C [22]. After being washed by centrifugation, the synaptosomes were resuspended in HBSS containing 1.0 mM Ca^{2+} and 0.5 mM Mg^{2+}. Aliquotes (200 μl) of synaptosomal samples were put into the 96 well plates with a final protein concentration 200-250 μg/ml. The fluorescence intensity was measured at 527 nm in response to 485 nm excitation with Thermo Scientific Fluoroskan Ascent equipped with fluorescent analysis system. The Ca^{2+} concentration is expressed as the fluorescence ratiometric $\Delta F/F_0\%$, which reflects a relative, not an absolute, measurement of the free Ca^{2+} concentration [23]. $\Delta F/F_0\% = (F-F_0)/F_0 \times 100$, where F is the fluorescence intensity at each time point and F_0 is the fluorescence intensity of the "rest" level given by the average of fluorescence intensities before adding different reagent.

2.6. Drugs

Ethyleneglycol-bis(β-aminoethyl ether) *N,N,N',N'*- tetraacetic acid (EGTA), 2-(4-morpholinoethyl-1)-phenylcyclohexane-1-carboxylate hydrochloride (PRE084), verapamil hydrochloride, nimodipine, chelerythrine, *N*-[2-(*p*-bromocinnamylamino)-ethyl]-5-isoquinolinesulfonamide dihydrochloride (H89), phorbol 12,13-dibutyrate (PDBu), AC915,

caffeine, (±)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrobromide (SKF38393), α-(4-fluorophenyl)-4-(S-fluoro-2-pyrimidinyl)-1-piperazinebutanol (BMY14802), cadmium chloride, rottlerin, 8-(4-chlorophenylthio)-adenosine-3',5'-cyclic monophosphate (CPT-cAMP), 3-isobutyl-1-methylxanthine (IBMX), 4-(3-butoxy-4-methoxybenzyl) imidazoline-2-one (Ro20-1724) and ω-conotoxin GVIA were purchased from Sigma. (+)-N-allylnormetazocine (SKF10047), thapsigargin, dizocilpine (MK801) and 5,6-bis[(4-fluorophenyl)amino]-1H-isoindole-1,3(2H)-dione (CGP53353) were from Tocris Bioscience. ω-Agatoxin IVA was from Alomone. Fluo-3 AM and pluronic F-127 were purchased from Molecular Probes. Non-radioactive PKA Assay Kit, non-radioactive AC assay Kit and non-radioactive PKC assay Kit were from Promega Corporation. Percoll was purchased from Amersham Biosciences Corporation. cPKCβI antibody was from Abcam. β-actin antibody was from Santa Cruz Biotechnology. Commercial sigma-1 antibody was from Invitrogen. The self-made sigma-1 receptor antibody was from Dr. Teruo Hayashi's lab. Other reagents in AR grades were products of Shanghai Chemical Plant. PRE084, EGTA, verapamil, H89, SKF10047, ω-Agatoxin IVA, ω-conotoxin GVIA, cadmium chloride, rottlerin, CPT-cAMP, SKF38393, BMY14802 and MK-801 were dissolved in ddH$_2$O and others were dissolved in DMSO. When DMSO was used as the vehicle, drugs were initially dissolved in 100% DMSO and then diluted into HBSS at a final DMSO concentration of 0.1%, which, we confirmed, had no detectable effects on the parameters we observed.

2.7. Off-line data analysis

Numerical data were expressed as mean ± SEM (standard error of means). Statistical significance was determined using Student's paired t-test for comparisons between two groups or ANOVA followed by Student–Newman–Keuls test for comparisons among three or more groups. In all cases n refers to the number of samples (different samples come from different animals) studied.

3. Results

3.1. Sigma-1 receptor agonists alone have no effects on PKA activity, but they can amplify the D1 receptor agonist-induced PKA activation

As shown in the left panel of Fig. 1A, the sigma-1 receptor agonist PRE084 [24] alone had no effects on the PKA activity (n = 5, P > 0.05), but it could significantly increase the effect of the D1 receptor agonist SKF38393 on the PKA activity in the synaptosomes from the prelimbic cortex. The PKA activity was increased by 30.5 ± 7.3% (n = 5, P < 0.05) at 4 min after SKF38393 (10 μM) alone, but after the synaptosomes were pretreated with PRE084 (10 μM) for 2 min, the PKA activity induced by SKF38393 was increased by 60.6 ± 10.3% (n = 5, P < 0.05, compared to the SKF38393 alone group). We also observed the influence of another sigma-1 receptor agonist SKF10047 [25] on the effect of SKF38393. In the SKF38393 alone group, SKF38393 was applied at 4 min before the measurement of PKA activity. In the SKF10047 + SKF38393 group, the synaptosomes were pretreated with SKF10047 for 2 min before SKF38393 and then SKF38393 was applied at 4 min before the measurement of PKA activity. The result showed that SKF10047 alone had no effects on the PKA activity (n = 4, P > 0.05, the right panel of Fig. 1B), but they could potentiate the effect of SKF38393 on the PKA activity (n = 4, the right panel of Fig. 1B). The PKA activity was enhanced by 19.1 ± 2.9% (n = 4, P < 0.05) by SKF38393 (10 μM) alone, but in the presence of SKF10047, the PKA activity induced by SKF38393 (10 μM) was increased by 58.6 ± 18.2% (n = 4, P < 0.05, compared to the SKF38393 alone group). Moreover, the potentiating effect of PRE084 on the effect of SKF38393 could be abolished by the sigma-1 receptor antagonist BMY14802 [26] (Fig. 1C, n = 4). In addition, we examined the expression of the sigma-1 receptors in the synaptosomes from the prelimbic cortex. The result

showed that the sigma-1 receptors were detected in the synaptosomes from the prelimbic cortex as a 25-kDa band in Western blotting (Fig. 1D). The commercial sigma-1 receptor antibody and the self-made sigma-1 receptor antibody from Dr. Teruo Hayashi showed a similar result. We also examined the expression of the sigma-1 receptors in the kidney tissues (Fig. 1D) as a control and obtained a similar result to that reported by Hayashi and Su [27]. Meanwhile, the synaptosomes incubated with the normal rabbit IgG showed no bands (Fig. 1D).

To explore the possible sites of the action of the sigma-1 receptor agonist in the D1-PKA signal transduction pathway, we observed the influence of the sigma-1 receptor agonist on the effect of the membrane-permeable analog of cAMP (CPT-cAMP) on the PKA activity. As shown in Fig. 2A, the PKA activity was enhanced by 75.0 ± 12.2% (n = 4, P < 0.05) by CPT-cAMP (1 μM) alone, but in the presence of PRE084 (10 μM), the PKA activity after CPT-cAMP was increased by 162.0 ± 16.1% (n = 4, P < 0.05, compared to the CPT-cAMP alone group). Also, the sigma-1 receptor agonist PRE084 could potentiate the effect of the adenylyl cyclase (AC) activator forskolin on the PKA activity (Fig. 2B). The PKA activity was enhanced by 36.1 ± 7.8% (n = 4, P < 0.05) by forskolin (20 μM) alone, but in the presence of PRE084 (10 μM), the PKA activity was increased by 64.8 ± 9.7% (n = 4, P < 0.05, compared to the forskolin alone group). However, PRE084 did not have the potentiating effect on the D1 receptor agonist SKF38393-induced AC activation (Fig. 2C). The AC activity was enhanced by 19.3 ± 4.7% (n = 8, P < 0.05) by SKF38393 (10 μM) alone and the AC activity was still increased by 18.8 ± 2.9% by SKF38393 (10 μM) in the presence of PRE084 (10 μM) (n = 8, P < 0.05, compared to control; P > 0.05, compared to SKF38393 group).

3.2. PKC plays an important role in the amplifying effect of the sigma-1 receptor agonist on the D1 receptor agonist-induced PKA activation

To test the role of PKC in the amplifying effect of the sigma-1 receptor agonist on the D1 receptor agonist-induced PKA activation, we observed the influence of the wide-spectrum PKC inhibitor chelerythrine [28] on the amplifying effect of the sigma-1 receptor agonist. The result showed that chelerythrine alone had no effects (n = 4), but the amplifying effect of PRE084 on the SKF38393-evoked PKA activation disappeared in the presence of chelerythrine (Fig. 3A, n = 5). This result suggests that the activation of PKC by PRE084 may play an important role in the amplifying effect of the sigma-1 receptor agonist on the D1 receptor agonist-induced PKA activation. This statement is further supported by our result that the PKC activator PDBu can mimic the amplifying effect of the sigma-1 receptor agonist on the D1 receptor agonist-induced PKA activation. The PKA activity was enhanced by 37.2 ± 18.1% (n = 4, P < 0.05) at 4 min after SKF38393 (10 μM) alone, but after the synaptosomes were pretreated with PDBu (1 μM) for 2 min, the PKA activity induced by SKF38393 was increased by 52.9 ± 22.3% (n = 4, P < 0.05, compared to the SKF38393 alone group).

To explore which isozymes of PKC were responsible for the amplifying effect of the sigma-1 receptor agonist on the D1 receptor agonist-induced PKA activation, we observed the influence of the selective PKC isozyme inhibitors on the effect of PRE084. We first observed the influence of rottlerin, a PKC inhibitor that was selective for the conventional PKC (cPKC) (including α, βI, βII and γ subtypes) at 30-42 μM [29], on the effect of PRE084. As shown in Fig. 3B, in the presence of rottlerin (40 μM), the amplifying effect of PRE084 on the SKF38393-evoked PKA activation disappeared. Rottlerin alone had no effects on the PKA activity (n = 4, P > 0.05). Next, we examined the influence of the cPKCβ-specific inhibitor CG53353 (the IC$_{50}$ value for cPKCβ, including cPKCβI and cPKCβII, is 3.8 μM and the IC$_{50}$ value for cPKCβII is 0.41 μM) [30] on the effect of PRE084. First, we used 4 μM CGP53353 to inhibit both cPKCβI and cPKCβII. The result showed that in the presence of 4 μM CGP53353, the amplifying effect of PRE084 on
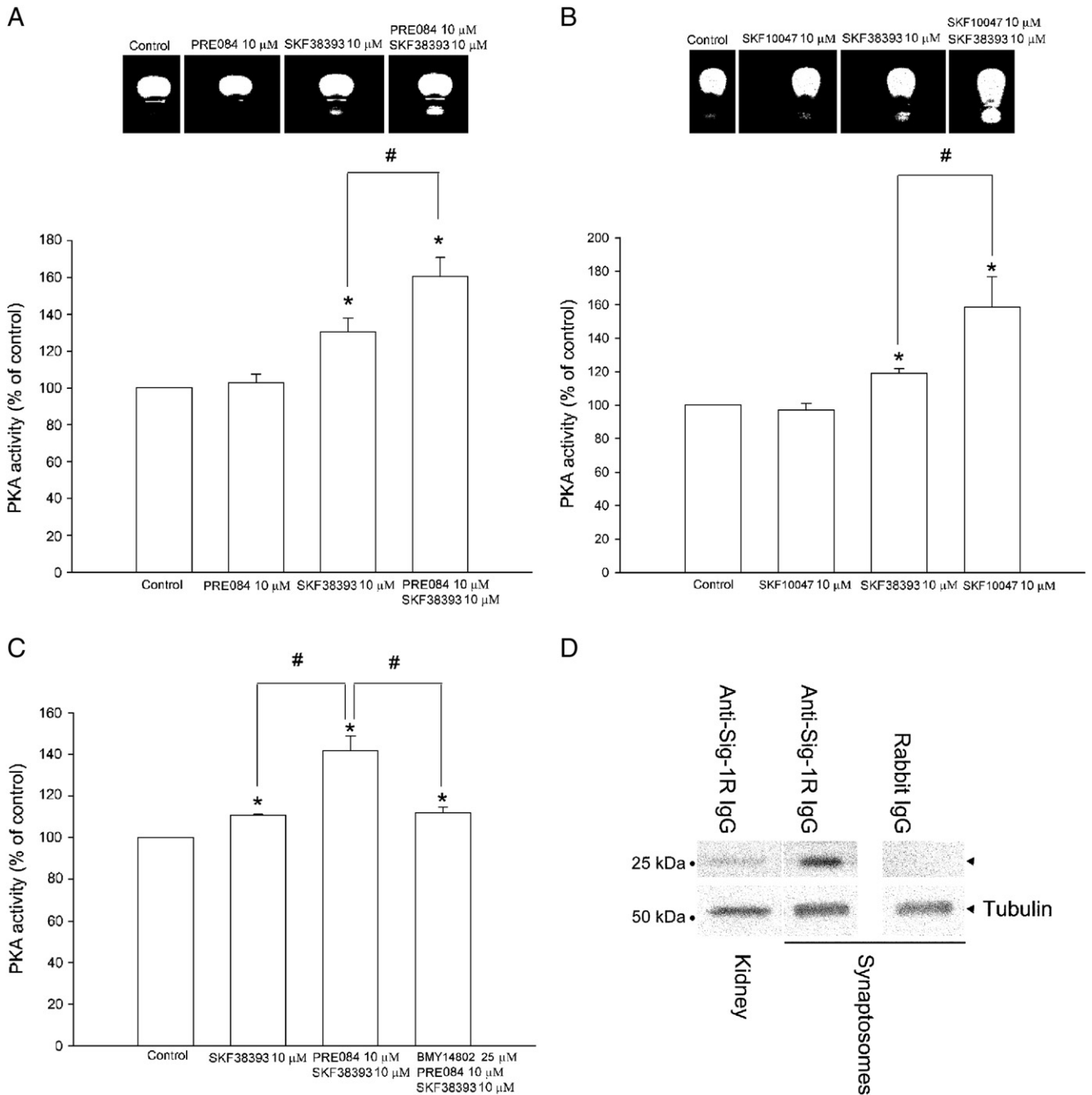
Fig. 1. Effect of the sigma-1 receptor agonists on the PKA activity and the D1 receptor agonist-induced PKA activation in the synaptosomes from the prelimbic cortex. A: The effect of the sigma-1 receptor agonist PRE084 (10 μM) on the PKA activity and the D1 receptor agonist SKF38393-induced PKA activation. In the SKF38393 alone group, SKF38393 (10 μM) was applied at 4 min before the measurement of PKA activity. In the PRE084 alone group, PRE084 (10 μM) was applied at 6 min before the measurement of PKA activity. In the PRE084 + SKF38393 group, the synaptosomes were pretreated with PRE084 for 2 min before SKF38393 and then SKF38393 was applied at 4 min before the measurement of PKA activity. Top figure: representative gel electrophoresis; Bottom figure: the averaged results in a group of animals. *P<0.05, compared to control; #P<0.05, compared to the SKF38393 alone group. n = 5. B: The effect of the sigma-1 receptor agonist SKF10047 on the PKA activity and the D1 receptor agonist SKF38393-induced PKA activation. In the SKF38393 alone group, SKF38393 (10 μM) was applied at 4 min before the measurement of PKA activity. In the SKF10047 alone group, SKF10047 (10 μM) was applied at 6 min before the measurement of PKA activity. In the SKF10047 + SKF38393 group, the synaptosomes were pretreated with SKF10047 for 2 min before SKF38393 and then SKF38393 was applied at 4 min before the measurement of PKA activity. Top figure: representative gel electrophoresis; Bottom figure: the averaged results in a group of animals. *P<0.05, compared to control; #P<0.05, compared to the SKF38393 alone group. n = 4. C: The influence of the sigma-1 receptor antagonist BMY14802 on the effect of PRE084. In the SKF38393 alone group, SKF38393 (10 μM) was applied at 4 min before the measurement of PKA activity. In the PRE084 + SKF38393 group, synaptosomes were treated by two-phase application of 2 min PRE084 (10 μM) and 4 min SKF38393 in sequence. In the BMY14802 + PRE084 + SKF38393 group, synaptosomes were treated with three-phase application of 2 min BMY14802 (25 μM), 2 min PRE084 and 4 min SKF38393 in sequence. *P<0.05, compared to control; #P<0.05, compared to the SKF38393 alone group or the PRE084 + SKF38393 group. n = 4. D: The expression of the sigma-1 receptors in the synaptosomes from the prelimbic cortex and the kidney tissues in Western blotting.

the SKF38393-evoked PKA activation disappeared (Fig. 3C, n=4). CGP53353 (4 μM) alone had no effects on the PKA activity (n=4, P>0.05). However, when we used 0.5 μM CGP53353, which was selective for cPKCβII, we did not find a significant influence of 0.5 μM

CGP53353 on the amplifying effect of PRE084 (Fig. 3D, n=4). CGP53353 (0.5 μM) alone had no effects on the PKA activity (n=4, P>0.05). We also checked the effect of PRE084 on the activity of cPKCβI with Western blot analysis. cPKCβI translocation from the

cytosolic to membrane fraction in synaptosomes was used to assess the relative amount of activated (membrane-bound) cPKCβI, an assay that was described previously [31]. cPKCβI activation is associated
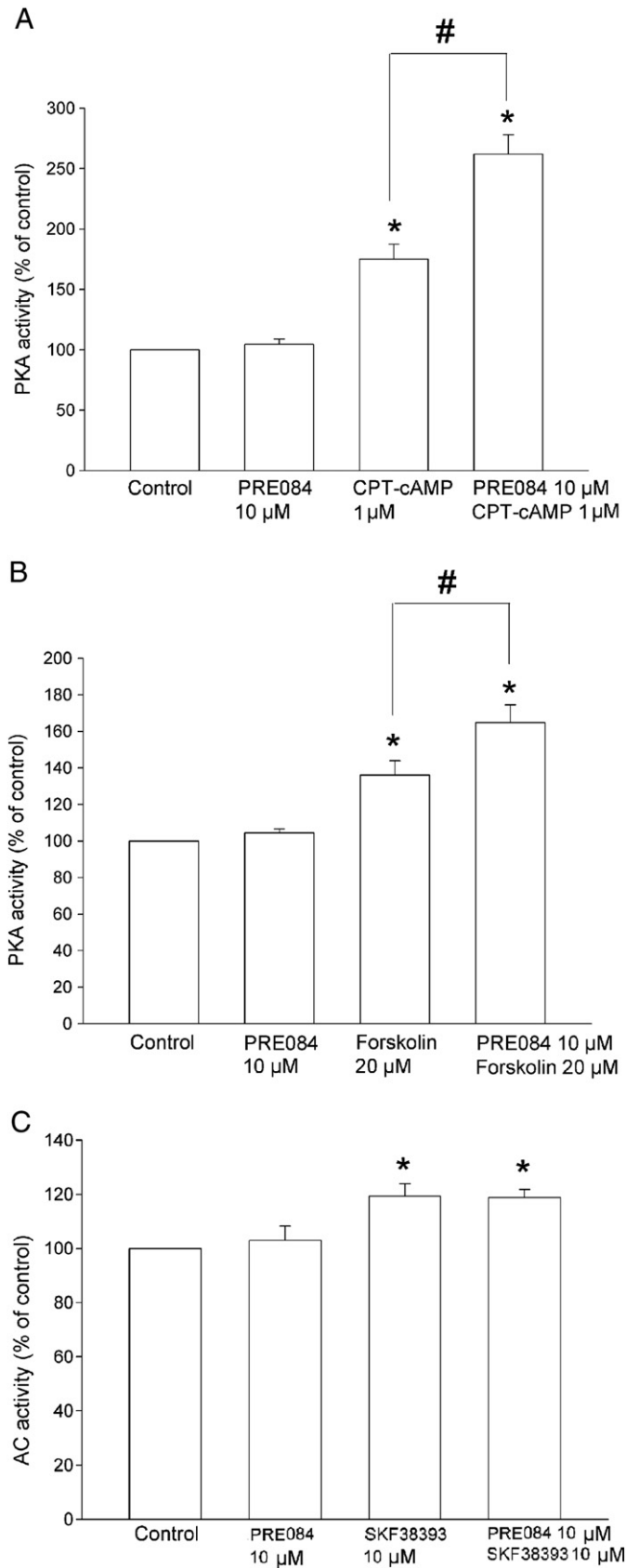
with translocation of the protein from the cytosol to cellular membranes. Fig. 4A showed representative immunoblots of the cPKCβI in both cytosolic and membrane fractions of synaptosomes before and 10 min after addition of PRE084 (10 μM) and the PKC activator PDBu (1 μM). Calculation of the percentage of cPKCβI in the membrane fraction revealed that PRE084 increased membrane-bound cPKCβI by 16.4% (Fig. 4B, n = 7, P<0.05), indicating that PRE084 stimulated the cPKCβI translocation. PDBu had a similar effect to that of PRE084 on the cPKCβI translocation (Fig. 4, n = 7, P<0.05).

3.3. Intrasynaptosomal Ca^{2+} plays an important role in the amplifying effect of the sigma-1 receptor agonist on the D1 receptor agonist-induced PKA activation

We observed the influence of the removal of extracellular Ca^{2+} by the Ca^{2+}-free medium containing EGTA [32] on the amplifying effect of the sigma-1 receptor agonist on the D1 receptor agonist-induced PKA activation. The result showed that the Ca^{2+}-free medium containing EGTA (1 mM) alone had no effects on the PKA activity (n = 4), but it could abolish the amplifying effect of PRE084 on the SKF38393-induced PKA activation (Fig. 5A, n = 4).

We also examined the influence of the voltage-gated Ca^{2+} channel blocker cadmium [33] on the amplifying effect of the sigma-1 receptor agonist. The result showed that cadmium (100 μM) alone had no effects (n = 4), but in the presence of cadmium, the amplifying effect of PRE084 disappeared (Fig. 5B, n = 4). To determine whether L-type Ca^{2+} channels were involved in the amplifying effect of PRE084, we observed the influence of the L-type Ca^{2+} channel blocker nimodipine [34] on the effect of PRE084. The results showed that nimodipine (10 μM) alone had no effects on the PKA activity (n = 4, P>0.05), but it could significantly inhibit the amplifying effect of PRE084 on the SKF38393-evoked PKA activation (n = 4, Fig. 5C).

3.4. Activation of PKC by sigma-1 receptor agonists is the upstream event of the increase in the intrasynaptosomal Ca^{2+} concentration

To study the relationship between the activation of PKC and the increase in the intrasynaptosomal Ca^{2+} concentration in the amplifying effect of the sigma-1 receptor agonist on the D1 receptor agonist-induced PKA activation, we observed the influence of the PKC inhibitor chelerythrine on the sigma-1 agonist-induced increase in the intrasynaptosomal Ca^{2+} concentration. The result showed that in the presence of chelerythrine, the effect of PRE084 on the intrasynaptosomal Ca^{2+} concentration disappeared (Fig. 6A). The averaged increase percentage of the Ca^{2+} concentration after PRE084 (10 μM) alone was 24.2 ± 1.7% (n = 6, P<0.05, compared to control), but in the

Fig. 2. Influence of the sigma-1 receptor agonist on the cAMP- and the AC activator-induced PKA activation and the effect of the sigma-1 receptor agonist on the D1 receptor agonist-induced AC activation in the synaptosomes from the prelimbic cortex. A: The influence of the sigma-1 receptor agonist PRE084 on the membrane-permeable analog of cAMP (CPT-cAMP)-induced PKA activation. In the cAMP alone group, cAMP (1 μM) was applied at 4 min before the measurement of PKA activity. In the PRE084 alone group, PRE084 (10 μM) was applied at 6 min before the measurement of PKA activity. In the PRE084 + cAMP group, synaptosomes were treated by two-phase application of 2 min PRE084 and 4 min cAMP in sequence. *P<0.05, compared to control; #P<0.05, compared to the CPT-cAMP alone group. n = 4. B: The influence of the sigma-1 receptor agonist PRE084 on the AC activator forskolin -induced PKA activation. In the forskolin alone group, forskolin (20 μM) was applied at 4 min before the measurement of PKA activity. In the PRE084 alone group, PRE084 (10 μM) was applied at 6 min before the measurement of PKA activity. In the PRE084 + forskolin group, synaptosomes were treated by two-phase application of 2 min PRE084 and 4 min forskolin in sequence. *P<0.05, compared to control; #P<0.05, compared to the forskolin alone group. n = 4. C: The effect of the sigma-1 receptor agonist PRE084 on the D1 receptor agonist SKF38393-induced AC activation. In the SKF38393 alone group, SKF38393 (10 μM) was applied at 4 min before the measurement of AC activity. In the PRE084 alone group, PRE084 (10 μM) was applied at 6 min before the measurement of AC activity. In the PRE084 + SKF38393 group, synaptosomes were treated by two-phase application of 2 min PRE084 and 4 min SKF38393 in sequence. *P<0.05, compared to control. n = 8.
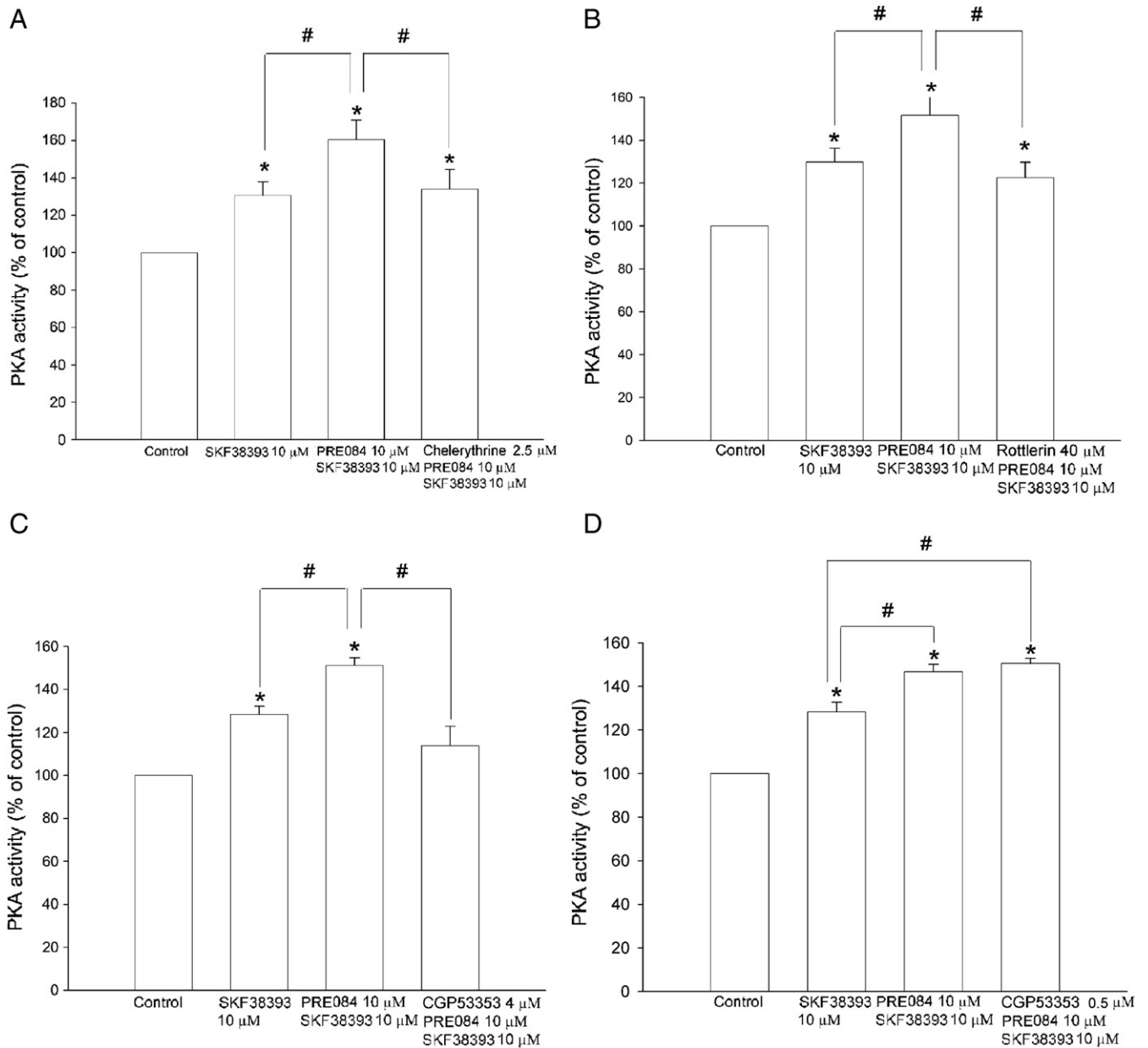
Fig. 3. Influence of PKC inhibitors on the amplifying effect of the sigma-1 receptor agonist on the D1 receptor agonist-induced PKA activation in the synaptosomes from the prelimbic cortex. A: Influence of the wide-spectrum PKC inhibitor chelerythrine on the amplifying effect of the sigma-1 receptor agonist PRE084. In the SKF38393 alone group, SKF38393 (10 µM) was applied at 4 min before the measurement of PKA activity. In the PRE084 + SKF38393 group, synaptosomes were treated by two-phase application of 2 min PRE084 (10 µM) and 4 min SKF38393 in sequence. In the chelerythrine + PRE084 + SKF38393 group, synaptosomes were treated with three-phase application of 2 min chelerythrine (2.5 µM), 2 min PRE084 and 4 min SKF38393 in sequence. *P<0.05, compared to control; #P<0.05, compared to the SKF38393 alone group or the PRE084 + SKF38393 group. n = 4. B: Influence of the cPKC inhibitor rottlerin on the amplifying effect of the sigma-1 receptor agonist PRE084. In the SKF38393 alone group, SKF38393 (10 µM) was applied at 4 min before the measurement of PKA activity. In the PRE084 + SKF38393 group, synaptosomes were treated by two-phase application of 2 min PRE084 (10 µM) and 4 min SKF38393 in sequence. In the rottlerin + PRE084 + SKF38393 group, synaptosomes were treated with three-phase application of 2 min rottlerin (40 µM), 2 min PRE084 and 4 min SKF38393 in sequence. *P<0.05, compared to control; #P<0.05, compared to the SKF38393 alone group or the PRE084 + SKF38393 group. n = 4. C: Influence of the cPKCβ inhibitor CGP53353 on the amplifying effect of the sigma-1 receptor agonist PRE084. In the SKF38393 alone group, SKF38393 (10 µM) was applied at 4 min before the measurement of PKA activity. In the PRE084 + SKF38393 group, synaptosomes were treated by two-phase application of 2 min PRE084 (10 µM) and 4 min SKF38393 in sequence. In the CGP53353 + PRE084 + SKF38393 group, synaptosomes were treated with three-phase application of 2 min CGP53353 (4 µM), 2 min PRE084 and 4 min SKF38393 in sequence. *P<0.05, compared to control; #P<0.05, compared to the SKF38393 alone group or the PRE084 + SKF38393 group. n = 4. D: Influence of the cPKCβII inhibitor CGP53353 (0.5 µM) on the amplifying effect of the sigma-1 receptor agonist PRE084. In the SKF38393 alone group, SKF38393 (10 µM) was applied at 4 min before the measurement of PKA activity. In the PRE084 + SKF38393 group, synaptosomes were treated by two-phase application of 2 min PRE084 (10 µM) and 4 min SKF38393 in sequence. In the CGP53353 + PRE084 + SKF38393 group, synaptosomes were treated with three-phase application of 2 min CGP53353 (0.5 µM), 2 min PRE084 and 4 min SKF38393 in sequence. *P<0.05, compared to control; #P<0.05, compared to the SKF38393 alone group or the PRE084 + SKF38393 group. n = 4.

presence of chelerythrine (2.5 µM), the percentage after PRE084 was 4.1 ± 1.5 % (n = 6, P>0.05, compared to control). Moreover, being consistent with the results of the above PKC isozyme experiments involving the mechanism of the amplifying effect of the sigma-1 receptor agonist on the D1 receptor agonist-induced PKA activation, both the cPKC inhibitor rottlerin (Fig. 6B, n = 6) and the cPKCβ

inhibitor CG53353 (4 µM) (Fig. 6C, n = 6) could also block the effect of PRE084 on the intrasynaptosomal Ca^{2+} concentration. In addition, the L-type Ca^{2+} channel blockers nimodipine (Fig. 7A, n = 6) and verapamil [35] (Fig. 7B, n = 6) could also abolish the effect of PRE084 on the intrasynaptosomal Ca^{2+} concentration. However, we did not find a significant influence of the N-type Ca^{2+} channel blocker
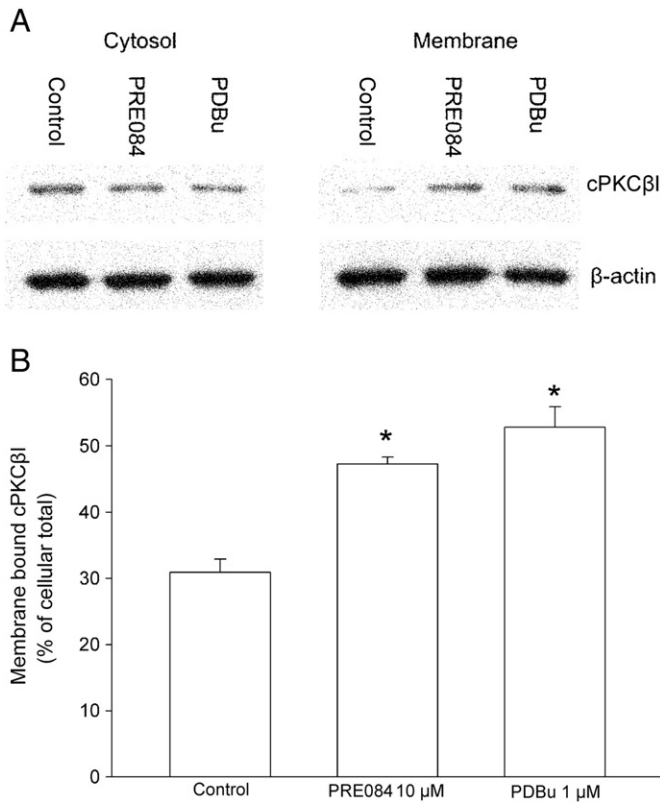
Fig. 4. Effect of PRE084 on the activity of cPKCβI using Western blot analysis in the synaptosomes from the prelimbic cortex. cPKCβI translocation from the cytosolic to membrane fraction in synaptosomes was used to assess the relative amount of activated (membrane-bound) cPKCβI. A: Representative immunoblots of the cPKCβI in both cytosolic and membrane fractions of synaptosomes before and 10 min after addition of PRE084 (10 μM) and PDBu (1 μM). B: Calculation of the percentage of cPKCβI in the membrane fraction before and 10 min after addition of PRE084 (10 μM) and PDBu (1 μM). *$P < 0.05$, compared to control. n = 7.

ω-contotoxin GVIA [36] (Fig. 7C, n = 6) and the P/Q-type Ca^{2+} channel blocker ω-agatoxin IVA [37] (Fig. 7D, n = 6) on the effect of PRE084 on the intrasynaptosomal Ca^{2+} concentration. We also did not find a significant influence of the NMDA receptor antagonist

Fig. 5. Influence of the extracellular Ca^{2+} chelator, the voltage-gated Ca^{2+} channel blocker and the L-type Ca^{2+} channel blocker on the amplifying effect of the sigma-1 receptor agonist on the D1 receptor agonist-induced PKA activation in the synaptosomes from the prelimbic cortex. A: Influence of the removal of the extracellular Ca^{2+} by the Ca^{2+}-free medium containing EGTA on the amplifying effect of the sigma-1 receptor agonist PRE084. In the SKF38393 alone group, SKF38393 (10 μM) was applied at 4 min before the measurement of PKA activity. In the PRE084 + SKF38393 group, synaptosomes were treated by two-phase application of 2 min PRE084 (10 μM) and 4 min SKF38393 in sequence. In the Ca^{2+}-free medium EGTA + PRE084 + SKF38393 group, synaptosomes were treated with three-phase application of 2 min Ca^{2+}-free medium containing EGTA (1 mM), 2 min PRE084 and 4 min SKF38393 in sequence. *$P < 0.05$, compared to control; #$P < 0.05$, compared to the SKF38393 alone group or the PRE084 + SKF38393 group. n = 4. B: Influence of the voltage-gated Ca^{2+} channel blocker cadmium on the amplifying effect of the sigma-1 receptor agonist PRE084. In the SKF38393 alone group, SKF38393 (10 μM) was applied at 4 min before the measurement of PKA activity. In the PRE084 + SKF38393 group, synaptosomes were treated by two-phase application of 2 min PRE084 (10 μM) and 4 min SKF38393 in sequence. In the cadmium + PRE084 + SKF38393 group, synaptosomes were treated with three-phase application of 2 min cadmium (100 μM), 2 min PRE084 and 4 min SKF38393 in sequence. *$P < 0.05$, compared to control; #$P < 0.05$, compared to the SKF38393 alone group or the PRE084 + SKF38393 group. n = 4. C: Influence of the L-type Ca^{2+} channel blocker nimodipine on the amplifying effect of the sigma-1 receptor agonist PRE084. In the SKF38393 alone group, SKF38393 (10 μM) was applied at 4 min before the measurement of PKA activity. In the PRE084 + SKF38393 group, synaptosomes were treated by two-phase application of 2 min PRE084 (10 μM) and 4 min SKF38393 in sequence. In the nimodipine + PRE084 + SKF38393 group, synaptosomes were treated with three-phase application of 2 min nimodipine (10 μM), 2 min PRE084 and 4 min SKF38393 in sequence. *$P < 0.05$, compared to control; #$P < 0.05$, compared to the SKF38393 alone group or the PRE084 + SKF38393 group. n = 4.
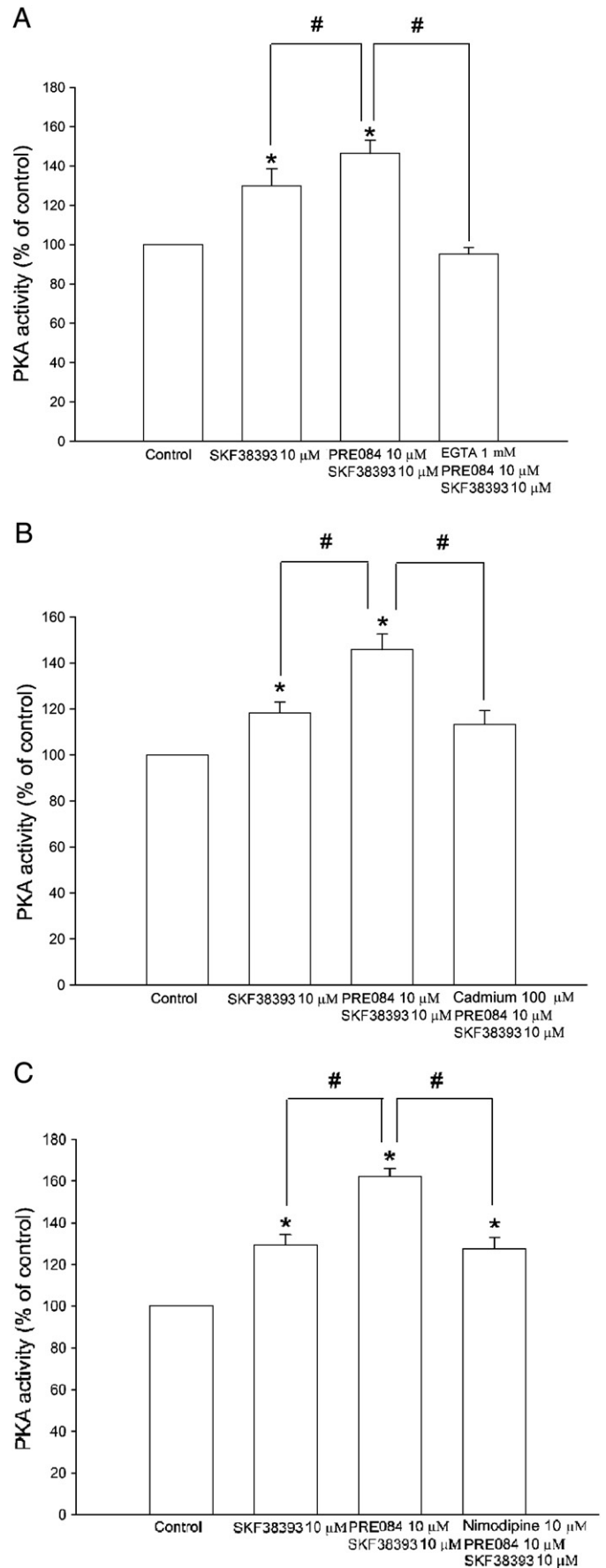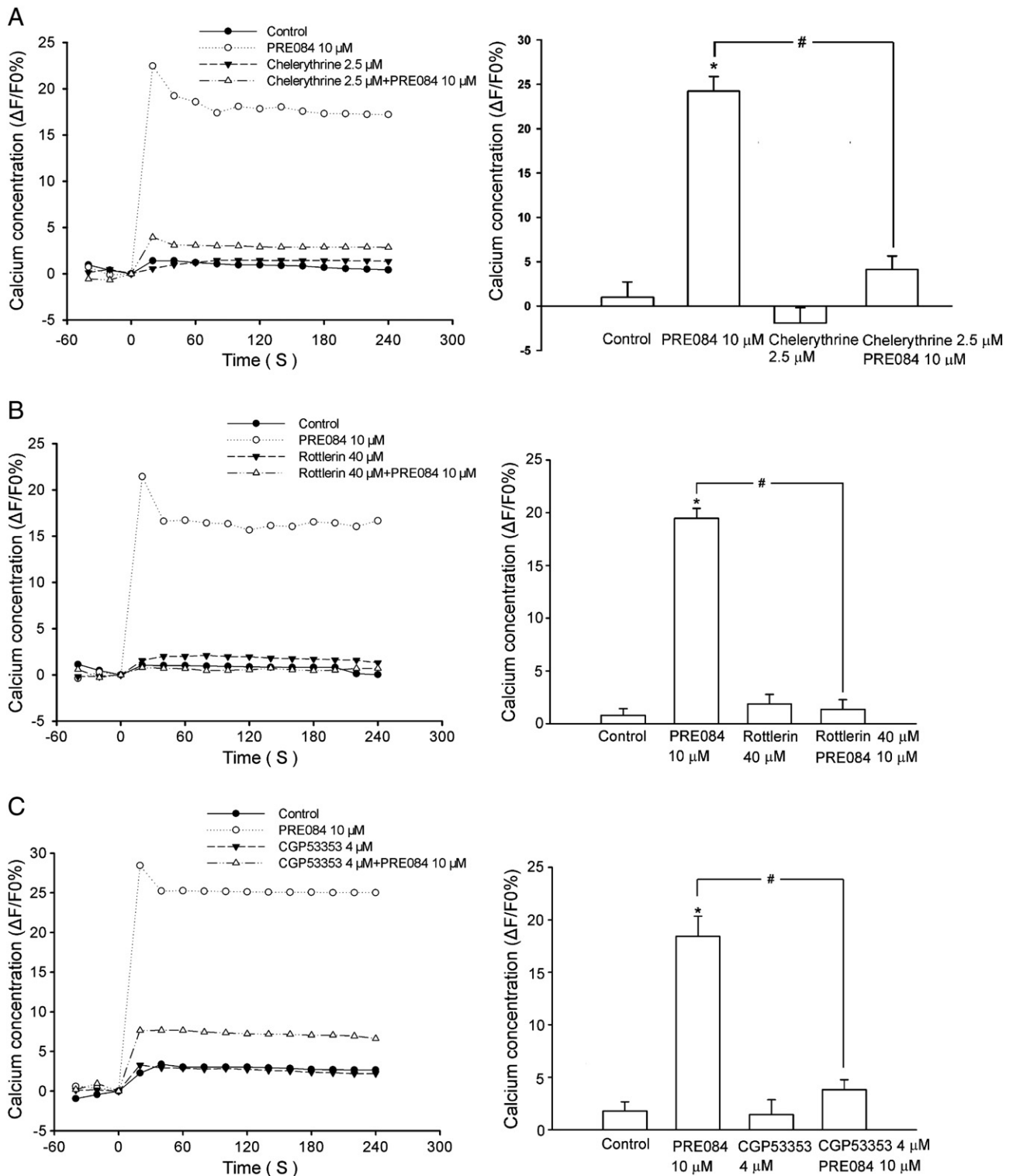
Fig. 6. Influence of PKC inhibitors on the effect of the sigma-1 receptor agonist on the intrasynaptosomal Ca^{2+} concentration in the synaptosomes from the prelimbic cortex. A: Influence of the wide-spectrum PKC inhibitor chelerythrine on the effect of the sigma-1 receptor agonist PRE084 on the intrasynaptosomal Ca^{2+} concentration. In the PRE084 alone group, PRE084 (10 μM) was applied at 4 min before the measurement of Ca^{2+} concentration. In the chelerythrine + PRE084 group, synaptosomes were treated by two-phase application of 2 min chelerythrine (2.5 μM) and 4 min PRE084 in sequence. *P<0.05, compared to control; #P<0.05, compared to the PRE084 alone group. n = 6. B: Influence of the cPKC inhibitor rottlerin on the effect of the sigma-1 receptor agonist PRE084 on the intrasynaptosomal Ca^{2+} concentration. In the PRE084 alone group, PRE084 (10 μM) was applied at 4 min before the measurement of Ca^{2+} concentration. In the rottlerin + PRE084 group, synaptosomes were treated by two-phase application of 2 min rottlerin (40 μM) and 4 min PRE084 in sequence. *P<0.05, compared to control; #P<0.05, compared to the PRE084 alone group. n = 6. C: Influence of the cPKCβ inhibitor CGP53353 (4 μM) on the effect of the sigma-1 receptor agonist PRE084 on the intrasynaptosomal Ca^{2+} concentration. In the PRE084 alone group, PRE084 (10 μM) was applied at 4 min before the measurement of Ca^{2+} concentration. In the CGP53353 + PRE084 group, synaptosomes were treated by two-phase application of 2 min CGP53353 (4 μM) and 4 min PRE084 in sequence. *P<0.05, compared to control; #P<0.05, compared to the PRE084 alone group. n = 6.

MK801 [38] on the effect of PRE084 on the intrasynaptosomal Ca^{2+} concentration (Fig. 7E, n=6). In addition, we also checked the role of intracellular Ca^{2+} stores in the sigma-1 receptor-mediated intrasynaptosomal Ca^{2+} increase. The result showed that thapsigargin, which depleted all intracellular Ca^{2+} stores by inhibiting the endosomal Ca^{2+}-ATPase activity [39], had no significant influence on the effect of PRE084. PRE084 (10 μM) still increased the intrasynaptosomal Ca^{2+} concentration by 22.6 ± 3.8% (n=6, P<0.05) in the presence of thapsigargin (25 μM), showing no significant

difference from that (22.9 ± 3.8%, n=6) of the PRE084 alone group (P>0.05).

4. Discussion

One of the main findings of the present study is that sigma-1 receptor agonists can amplify the D1 receptor agonist-induced PKA activation at presynaptic sites in the prelimbic cortex. To the best of
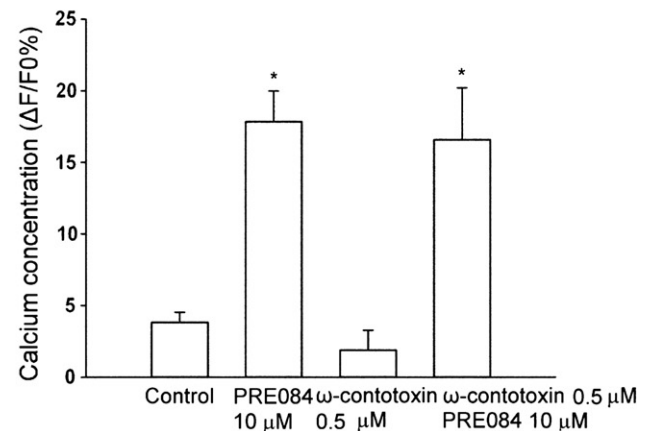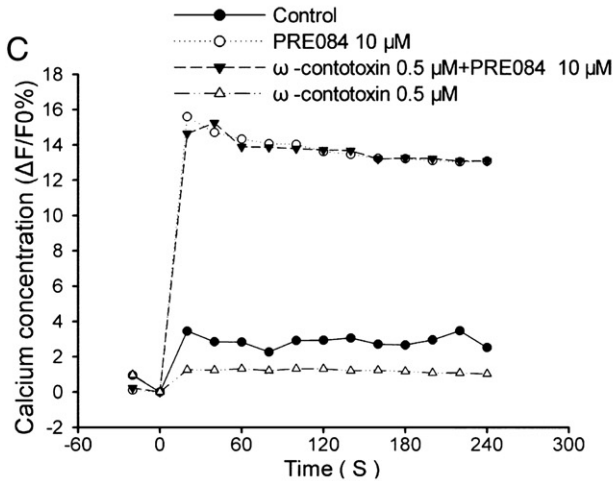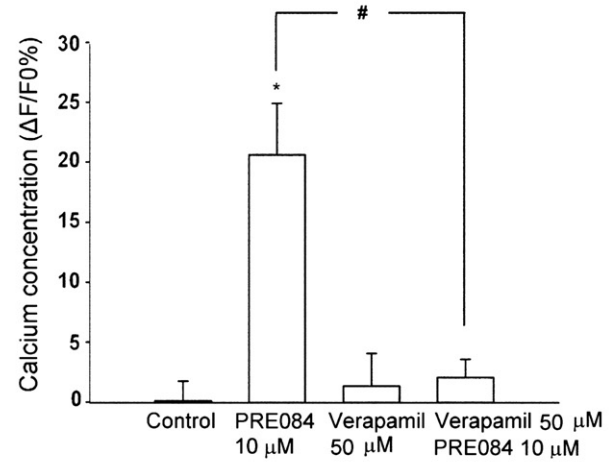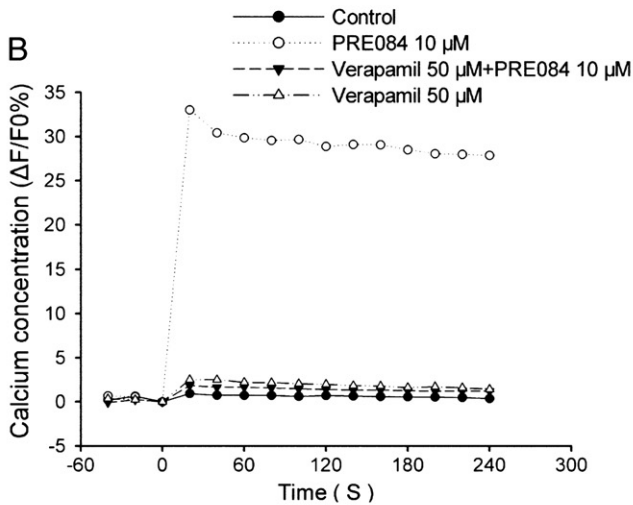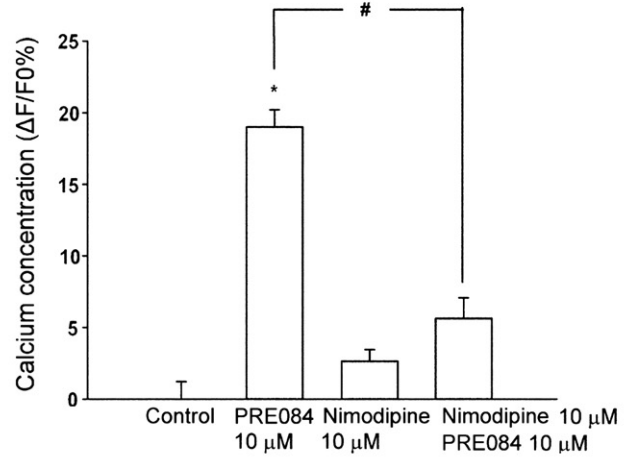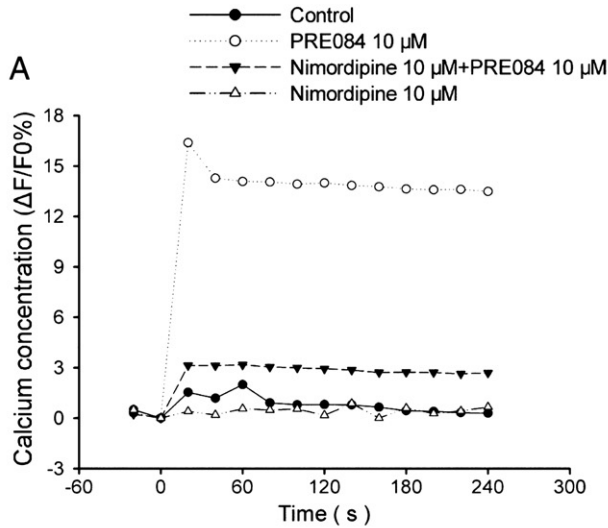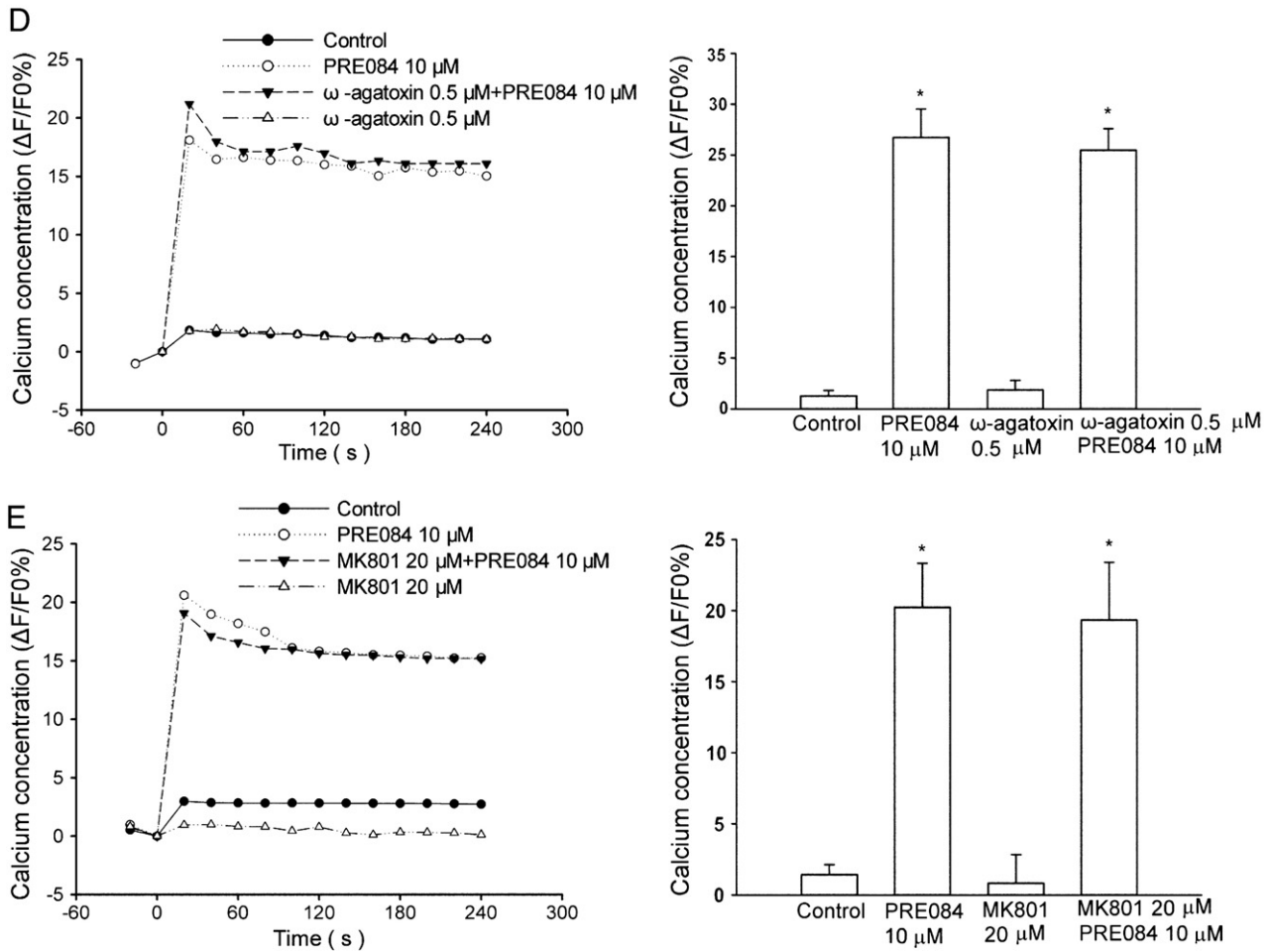
Fig. 7. Influence of the L-type Ca^{2+} channel blockers, the N-type Ca^{2+} channel blocker, the P/Q-type Ca^{2+} channel blocker and the NMDA receptor antagonist on the effect of the sigma-1 receptor agonist on the intrasynaptosomal Ca^{2+} concentration in the synaptosomes from the prelimbic cortex. A: The influence of the L-type Ca^{2+} channel blocker nimodipine on the effect of the sigma-1 receptor agonist PRE084 on the intrasynaptosomal Ca^{2+} concentration. In the PRE084 alone group, PRE084 (10 μM) was applied at 4 min before the measurement of Ca^{2+} concentration. In the nimodipine + PRE084 group, synaptosomes were treated by two-phase application of 2 min nimodipine (10 μM) and 4 min PRE084 in sequence. *P<0.05, compared to control; #P<0.05, compared to the PRE084 alone group. n = 6. B: The influence of the L-type Ca^{2+} channel blocker verapamil on the effect of the sigma-1 receptor agonist PRE084 on the intrasynaptosomal Ca^{2+} concentration. In the PRE084 alone group, PRE084 (10 μM) was applied at 4 min before the measurement of Ca^{2+} concentration. In the verapamil + PRE084 group, synaptosomes were treated by two-phase application of 2 min verapamil (50 μM) and 4 min PRE084 in sequence. *P<0.05, compared to control; #P<0.05, compared to the PRE084 alone group. n = 6. C: The influence of the N-type Ca^{2+} channel blocker ω-contotoxin GVIA on the effect of the sigma-1 receptor agonist PRE084 on the intrasynaptosomal Ca^{2+} concentration. In the PRE084 alone group, PRE084 (10 μM) was applied at 4 min before the measurement of Ca^{2+} concentration. In the ω-contotoxin GVIA + PRE084 group, synaptosomes were treated by two-phase application of 2 min ω-contotoxin GVIA (0.5 μM) and 4 min PRE084 in sequence. *P<0.05, compared to control. n = 6. D: The influence of the P/Q-type Ca^{2+} channel blocker ω-agatoxin IVA on the effect of the sigma-1 receptor agonist PRE084 on the intrasynaptosomal Ca^{2+} concentration. In the PRE084 alone group, PRE084 (10 μM) was applied at 4 min before the measurement of Ca^{2+} concentration. In the ω-agatoxin IVA + PRE084 group, synaptosomes were treated by two-phase application of 2 min ω-agatoxin IVA (0.5 μM) and 4 min PRE084 in sequence. *P<0.05, compared to control. n = 6. E: The influence of the NMDA receptor antagonist MK801 on the effect of the sigma-1 receptor agonist PRE084 on the intrasynaptosomal Ca^{2+} concentration. In the PRE084 alone group, PRE084 (10 μM) was applied at 4 min before the measurement of Ca^{2+} concentration. In the MK801 + PRE084 group, synaptosomes were treated by two-phase application of 2 min MK801 (20 μM) and 4 min PRE084 in sequence. *P<0.05, compared to control. n = 6.

our knowledge, this is the first report involving the regulation of D1 receptor signaling by sigma-1 receptors.

The concentration (10 μM) of PRE084 used here to produce the effect was larger than the K_i value (44 nM) of PRE084 [40], but this effect should be specific to the sigma-1 receptors. The evidence supporting this statement was that (1) the specific sigma-1 receptor antagonist BMY14802 could completely abolish the effect of PRE084; (2) in the literature, although the Ki value of PRE084 for the sigma-1 receptors was in the nanomolar range, the IC_{50} values of PRE084 for producing effects via the activation of the sigma-1 receptors were generally in the micromoles range. For example, the IC_{50} value of PRE084 for the inhibition of the ASIC1a-induced $[Ca^{2+}]i$ increase was 13.7 μM [41] and for the protection of human retinal cells against oxidative stress was 10 μM [42].

Sigma-1 receptors have been known to exist at postsynaptic sites. In addition, a number of evidence suggested that sigma-1 receptors might also exist at presynaptic sites. The evidence supporting the existence of sigma-1 receptors at presynaptic sites was that (1) the functional experiments in brain slices [43–45] and synaptosomes (a preparation of presynaptic terminals) [46] showed that the activation of sigma-1 receptors could modulate presynaptic neurotransmitter release; (2) radioligand binding experiments showed that selective sigma-1 receptor agonists could bind with synaptosomes with high affinity in a competitive manner [47]. Moreover, the present study showed the existence of sigma-1 receptors in the synaptosomes by Western blotting.

The signaling pathway between D1 receptors and PKA involves the coupling to Gs-protein, the activation of AC and the action of cAMP on

PKA. We explored the possible sites of the action of the sigma-1 receptors in this pathway. First, we used CPT-cAMP, a membrane-permeable analog of cAMP, to generate the cAMP-induced PKA activation and then observed the influence of the sigma-1 receptor agonist on this activation. The result showed that the sigma-1 receptor agonist could potentiate the effect of CPT-cAMP on PKA. Then, we used forskolin, an activator of AC, to generate the AC/cAMP-induced PKA activation and observed the influence of the sigma-1 receptor agonist on this activation. The result showed that the sigma-1 receptor agonist could also potentiate this activation. However, we did not find that the sigma-1 receptor agonist had the potentiating effect on the D1 receptor agonist-induced AC activation. These results suggest that the sigma-1 receptor agonist may amplify D1 receptor signaling via amplifying the action of cAMP on PKA.

It has been known that the downstream pathway of the sigma-1 receptors involves the activation of PKC and the enhancement of intracellular Ca^{2+} concentration. Therefore, it is possible that PKC and intracellular Ca^{2+}, as important downstream signaling molecules of sigma-1 receptors, play an important role in the amplifying effect of the sigma-1 receptor agonist on the D1 receptor agonist-induced PKA activation. This hypothesis has been confirmed by our results. Moreover, this result provides evidence supporting that sigma-1 receptors may amplify D1 receptor signaling via PKC and intracellular Ca^{2+}.

Noteworthily, when we removed the extracellular Ca^{2+} by the Ca^{2+}-free medium containing EGTA, the activity of PKA returned to the control levels but cadmium and nimodipine only inhibited the amplifying effect achieved by PRE084. This phenomenon suggests possible involvement of another Ca^{2+} source in the D1 receptor-induced PKA activation. In this aspect, it's possible that this another Ca^{2+} source may be from D-1 receptor-induced intracellular Ca^{2+} release because it is shown that the activation of D1 receptors can result in a rise in intracellular Ca^{2+} through both extracellular influx and release from intracellular compartments [48].

PKC family includes at least 12 isozymes, which are classified into three major groups [49]. The first of these groups is the conventional PKC (cPKC), which includes α, βI, βII and γ types. The second group, called the novel PKC (nPKC), includes δ, ε, η and θ type. The third group is the atypical PKC (aPKC), including ζ and λ type. The PKC isozymes are distributed in various tissues. Among them, cPKC is the one localized at presynaptic terminals [50]. So it is possible that cPKC plays an important role in the amplifying effect of the sigma-1 receptor agonist on the D1 receptor agonist-induced PKA activation. This hypothesis was confirmed by our result that the cPKC selective inhibitor rottlerin could abolish the effect of the sigma-1 receptor agonist. Moreover, CG53353 at 4 μM, which inhibited both PKCβII and PKCβI of cPKC, could also abolish the effect of the sigma-1 receptor agonist. However, CGP53353 at 0.5 μM, which was selective for PKCβII, had no influence. These results suggest that the cPKC, especially the PKCβI, may play a key role in the effect of the amplifying effect of the sigma-1 receptor agonist on the D1 receptor agonist-induced PKA activation.
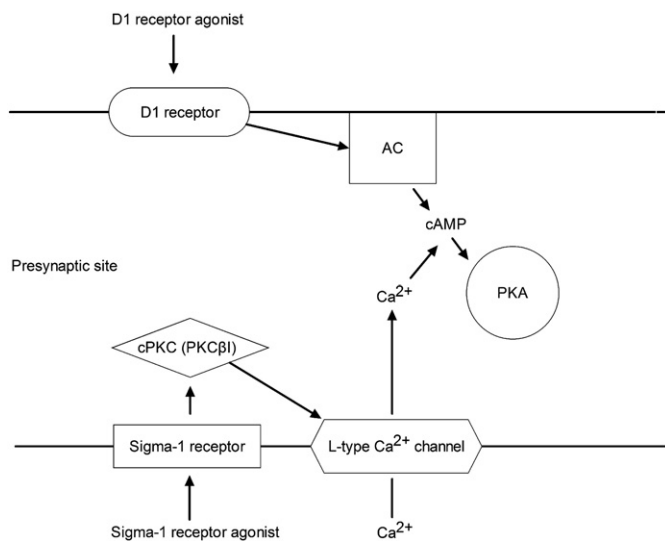
About the source of Ca^{2+} involving the amplifying effect of the sigma-1 receptor agonist on the D1 receptor agonist-induced PKA activation, it may involve the promotion of the extracellular Ca^{2+} influx or the mobilization of the intracellular Ca^{2+} store. In the present paper, we studied the role of the extracellular Ca^{2+} in the effect of the sigma-1 receptor agonist. The result showed that after removing the extracellular Ca^{2+} by the Ca^{2+}-free medium containing EGTA, the amplifying effect of the sigma-1 receptor agonist disappeared. Moreover, the voltage-gated Ca^{2+} channel blocker cadmium and the L-type Ca^{2+} channel blocker nimodipine could abolish the amplifying effect of the sigma-1 receptor agonist, suggesting that the extracellular Ca^{2+} influx through the L-type Ca^{2+} channels might mediate the amplifying effect of the sigma-1 receptor agonist.

About the relationship between the activation of PKC and the increase in the intrasynaptosomal Ca^{2+} concentration in the ampli-

fying effect of the sigma-1 receptor agonist on the D1 receptor agonist-induced PKA activation, our results suggested that the activation of PKC by sigma-1 receptor agonists might be the upstream event of the increase in the intrasynaptosomal Ca2+ concentration. Interestingly, based on our results, it appeared that the sigma-1 receptors could modulate the resting-state L-type Ca^{2+} channels, which then led to an increase in the intrasynaptosomal Ca^{2+} concentration. This statement was consistent with the evidence: (1) there were spontaneous L-type Ca^{2+} channel activities at the rest membrane potentials [51] and these activities contributed to the genesis of spontaneous Ca^{2+} sparks in the cytoplasm [52]; (2) some active substances, such as ganglioside and phorbol ester (the PKC activator), could increase the Ca^{2+} influx through the modulation of these activities [53,54]. However, how sigma-1 receptors modulate the resting-state L-type Ca^{2+} channels remains to be studied. In addition, previous studies in NG-108 and SHSY5Y cell lines showed that the sigma-1 receptor agonists enhanced the intracellular Ca^{2+} by the mobilization of the intracellular Ca^{2+} store [55–57]. However, this mechanism might not be involved in the effect of the sigma-1 receptor agonists on the intrasynaptosomal Ca^{2+} concentration in the prelimbic cortex because our result showed that the intracellular Ca^{2+} store depleter had no significant influence on the effect of the sigma-1 receptor agonist. Moreover, the present result that the removal of the extracellular Ca^{2+} by the Ca^{2+}-free medium containing EGTA could abolish the effect of the sigma-1 receptor agonist also supported that the mechanism of the effect of the sigma-1 receptor agonist on the intrasynaptosomal Ca^{2+} concentration might involve the promotion of the extracellular Ca^{2+} influx, rather than the mobilization of the intracellular Ca^{2+} store.

Subcellular distribution of sigma-1 receptors has been studied with radioligand binding in subcellular fractions, and more recently with immunochemical methods. These studies found that sigma-1 receptors were abundant in endoplasmic reticulum (ER), nuclear, mitochondrial and synaptic membranes (synaptosomes) [3]. However, it appears that the functions of sigma-1 receptors in different subcellular fractions are different. For example, recent studies show that the sigma-1 receptors in the ER, as a Ca^{2+}-sensitive and ligand-operated receptor chaperone at mitochondrion-associated ER membrane (MAM), can lead to a prolonged Ca^{2+} signaling into the mitochondria via IP3 receptors, and thus regulating ER-mitochondrial interorganellar Ca^{2+} signaling and cell survival [58]; the present study shows that one function of the sigma-1 receptors at presynaptic sites (synaptosomes) may amplify the D1 receptor agonist-induced PKA activation by the sigma-1 receptors-cPKC (especially the PKCβI)- L-type Ca^{2+} channels-Ca^{2+}-AC and/or cAMP signaling pathway (Fig. 8). It is noteworthy that the synaptosomal preparation may be contaminated with ER or mitochondrion where sigma-1 receptors are highly concentrated in a specialized area known as the MAM. But this contamination may not contribute to the amplifying effect of the sigma-1 receptor agonist on the D1 receptor agonist-induced PKA activation because these contaminated ER or mitochondria are in the extrasynaptosomal space.

Significance – It has been known that the dopaminergic projection from the ventral tegmental area to the prelimbic cortex plays a major role in cognition and neuropsychiatric processes. In particular, the involvement of prefrontal D1 dopamine receptors in the behavioral sensitization to psychostimulants has received much attention. The activation of D1 receptors in the prelimbic cortex stimulated psychostimulant-induced motor activity and increased behavioral sensitization to psychostimulants [59–62]. Thus, the present finding that the sigma-1 receptors can amplify the presynaptic dopamine D1 receptor signaling in the prelimbic cortex is of significance for understanding the neuronal basis of the behavioral sensitization induced by sigma-1 receptors. In addition, the present finding that the activation of sigma-1 receptors amplifies the D1 receptor-induced PKA activation via cPKC (especially the PKCβI) - L-type Ca^{2+} channels - Ca^{2+} - AC and/or cAMP signaling pathway is of important significance

Fig. 8. Schematic representation of the possible mechanism underlying the amplifying effect of the sigma-1 receptor agonist on the D1 receptor agonist-induced PKA activation in the synaptosomes from the prelimbic cortex. The sigma-1 receptor in the presynaptic site may amplify the D1 receptor agonist-induced PKA activation by sigma-1 receptors - cPKC (especially the PKCβI) - L-type Ca^{2+} channels - Ca^{2+} - AC and/or cAMP signaling pathway.

for revealing the downstream signal transduction pathway of sigma-1 receptors.

Acknowledgements

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