Cyclosporine attenuates the adenylyl cyclase superactivation induced by chronic cannabinoid treatment

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Received 13 September 2006; received in revised form 6 November 2006; accepted 8 November 2006
Available online 14 November 2006

Abstract

Chronic cannabinoid treatment results in the development of tolerance. Adenylyl cyclase superactivation, induced by chronic cannabinoid agonist administration, is regarded as one of the molecular mechanisms leading to tolerance. In the present study, the effect of cyclosporine on adenylyl cyclase superactivation after chronic exposure to WIN 55,212-2, a cannabinoid receptor agonist, was studied. Chronic treatment (18 h) with WIN 55,212-2 induced a significant increase in cAMP levels in human astrocytoma cells (adenylyl cyclase superactivation). Acute treatment with cyclosporine (10 min) did not have any effect on WIN 55,212-2-induced adenylyl cyclase superactivation. But, chronic cyclosporine treatment (18 h), with concentration from 1 nM to 1 μM, attenuates the development of adenylyl cyclase superactivation after chronic WIN 55,212-2 treatment. Our findings show that cyclosporine attenuates chronic cannabinoid-mediated adenylyl cyclase super activation.

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Keywords: Cannabinoid; Adenylyl cyclase superactivation; Cyclosporine

1. Introduction

It is now well established that cannabinoids mediate their cellular effect through specific cannabinoid receptors. Two subtypes of cannabinoid receptors have been cloned, CB1 and CB2. Both receptors belong to the seven transmembrane domain GTP-binding protein (G protein)-coupled receptor superfamily (Matsuda et al., 1990; Munro et al., 1993). It has been demonstrated that both types of cannabinoid receptors act via Gi/o to inhibit adenylyl cyclase (AC) (Howlett, 1995), to inhibit voltage-gated N- and Q-type Ca2+ channels and to active voltage-sensitive K+ channels (Mackie et al., 1995).

Chronic cannabinoid treatment results in the development of behavioral tolerance and dependence. But, the cellular and molecular mechanisms of cannabinoid tolerance are not entirely clear. Pervious studies suggest that a compensatory increase in the adenylyl cyclase activity (adenylyl cyclase superactivation) in response to chronic cannabinoid receptor stimulation, may contribute to cellular cannabinoid tolerance (Rhee et al., 2000).

Cyclosporine (cyclosporin A) is a powerful suppressor of the immune system, widely used to prevent rejection of transplanted organs. The immunosuppressive effect of cyclosporine is due to binding to its protein receptors, immunophilins (Handsenschumacher et al., 1984). Recently, it has been reported that immunophilins are more abundant in the nervous system than immune system (Steiner et al., 1992). Subsequently some important actions of immunophilin-binding ligands in the nervous system have been revealed, which include regulation of neurotransmitter release, neurotrophic effects, regulation of intracellular calcium release and inhibition of nitric oxide (NO) synthesis (Synder et al., 1998). Recently, our group showed that cyclosporine can attenuate cannabinoid (Banafshe et al., 2005) and morphine tolerance in mice (Homayoun et al., 2002) and guinea pig ileum model (Ejtemaei Mehr et al., 2003). Further support for the possible effect of cyclosporine on cannabinoid-mediated adenylyl cyclase superactivation is provided by Gary-Gouy et al. (2006) that cyclosporine indirectly inhibited extracellular signal-regulated protein kinase (ERK) activation trough sequestration of Raf-1. It was also reported that morphine-mediated adenylyl cyclase superactivation is attenuated by Raf-1 inhibitors (Yue et al., 2006). Cannabinoids and opioids have many similar pharmacological properties, common signaling mechanisms and cross
tolerance (Shapira et al., 2003). These similarities between opioid and cannabinoid raise the possibility of the effect of cyclosporine on cannabinoid-mediated adenylyl cyclase superactivation.

2. Materials and methods

A cannabinoid receptor agonist R(+-)[2,3-Dihydro-5-methyl-3[(morpholinyl) methyl] pyrrolo[1,2,3-de]-1,4-benzoazin-yl]-(1-naphthalenyl) methane mesylate (WIN 55,212-2) and a cannabinoid antagonist N-(piperidin-1-yl)-1-(2,4-dichlorophenyl)-5-(4iodophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM 251) were purchased from Tocris Cookson (Bristol, UK), Cyclosporine from Sandoz Pharma (Basel, Switzerland). Forskolin, Dulbecco’s Modified Eagle Medium (DMEM) and Fetal Calf Serum were from Sigma (St.Louis, USA). cAMP enzyme immunoassay (EIA) system kit from Amersham Pharmacia Biotech (little Chalfont, UK). The human astrocytoma cell line U373 MG (ATCC, HTB-17) was obtained from National Cell Bank of Iran (Tehran, Iran).

The cells were grown in DMEM supplemented with 10% fetal calf serum, 2 mM glutamine, 100 unit/ml penicillin and streptomycin at 37 °C in a humidified atmosphere of 5% CO2/95% air. Cell was grown in 75 cm² flasks until reaching confluency and after conventional trypsinization procedure, cells were counted and plated in 96 multiwell plates at density of 2×10⁵ cells/well and allowed to adhere 24 h at 37 °C and then drugs were incubated for specific time periods.

First, the effects of acute (10 min) and chronic (18 h) treatment with WIN 55,212-2 on the forskolin-stimulated adenylyl cyclase activity in human astrocytoma cell line (U373 MG) were studied. Then, to study the effect of cyclosporine on cannabinoid-mediated adenylyl cyclase activity in human astrocytoma cell line (U373 MG) were studied. Then, to study the effect of cyclosporine on cannabinoid-mediated adenylyl cyclase superactivation, cells were preincubated with WIN 55,212-2 (1 μM) for 18 h (Rhee et al., 2000). Cyclosporine was incubated for 10 min (acute treatment) or for 18 h before the 10-minute assay (started by the addition of forskolin) (chronic treatment) (Rhee et al., 2000). The assay was performed in triplicate by using PRN 225, Biotrak cAMP enzyme immunoassay (EIA) system kit from Amersham Pharmacia Biotech. All experiments were repeated twice. The data were analyzed using Graphpad Prism data analysis program. A one-way analysis of variance (ANOVA) followed by Turkey’s test was used. In a few cases in which only two groups were to be compared, Student’s t-test was used.

3. Results

As shown in Fig. 1. Acute treatment (10 min) with WIN 55,212-2 induced inhibition of forskolin-stimulated adenylyl cyclase activity with an EC₅₀ of 1.85±0.45 nM and Eₘₐₓ of 58±2.5% in U 373 MG cell line. In chronic exposure to WIN 55,212-2, a significant increase in adenylyl cyclase activity (adenylyl cyclase superactivation) was observed in cells treated for 18 h with 1 to 1000 nM WIN 55,212-2 followed by withdrawal of agonist before the assay (Fig. 1). The cannabinoid receptor antagonist, AM251 (1 μM), completely prevented cannabinoid-mediated cAMP overshoot (data not shown) indicating that cannabinoid receptor stimulation is necessary for the development of AC superactivation.

In acute treatment with cyclosporine (10 min), any used concentration did not change the chronic cannabinoid-mediated increase in forskolin-stimulated cAMP formation (data not shown) but, chronic treatment (18 h) of U3732 MG cells with cyclosporine with concentration from 1 nM to 1 μM, attenuates the development of adenylyl cyclase superactivation induced by chronic WIN 55,212-2 treatment, F(5,30)=50.056 P<0.001 (Fig. 2).

4. Discussion

In the present study, we demonstrate that, chronic treatment with cyclosporine attenuates cannabinoid-mediated adenylyl cyclase (AC) superactivation in human astrocytoma cell line.
Recently, we have reported that systemic administration of cyclosporine can attenuate the development of tolerance to WIN 55,212-2 in mice (Banafshe et al., 2005) and adenylyl cyclase superactivation after chronic cannabinoid agonist exposure is thought to play an important role in cannabinoid tolerance and dependence (Rhee et al., 2000; Rubino et al., 2000). Therefore, we decide to study the effect of cyclosporine on adenylyl cyclase superactivation after chronic cannabinoid treatment.

In agreement with the previous results (Dill and Howlett, 1988; Rhee et al., 2000), we showed that chronic treatment (18 h) with WIN 55,212-2 caused AC superactivation in human astrocytoma cell line.

The fact that acute treatment with cyclosporine could not affect cannabinoid-mediated AC superactivation is in agreement with our previous study (Banafshe et al., 2005) that shown acute treatment with cyclosporine did not have any effect on cannabinoid tolerance in mice. Attenuation of cannabinoid-mediated AC superactivation by chronic cyclosporine treatment may indicate that cyclosporine act through interference with underlying pathways of development of AC superactivation.

Phosphorylation of Ser/Thr residues in the vicinity of the catalytic domain of AC isoenzymes is an important molecular mechanism to regulate their catalytic activity. Thus, it was shown that protein kinase Raf-1 directly binds and phosphorylates several AC isoenzymes, leading to their sensitization towards different stimulators, such as forskolin or activated Gs proteins (Tan et al., 2001; Ding et al., 2004). Recently, Yue et al. (2006) have found that chronic morphine-mediated adenylyl cyclase superactivation is attenuated by the Raf-1 inhibitor. Interestingly, it was also demonstrated that cyclosporine inhibits the mitogen-activated protein kinase (MAP kinase) activation through sequestration of Raf-1 (Gary-Gouy et al., 2006).

In addition, it was shown that cyclosporine inhibits protein kinase C activity and calcium/calmodulin-dependent protein phosphatase (Walker et al., 1989; Groblewski et al., 1994) and it was also reported that protein kinase C and calmodulin are involved in the pathway leading to chronic opioid-mediated AC superactivation (Varga et al., 2003). Therefore, cyclosporine may attenuate cannabinoid-mediated AC superactivation through sequestration of Raf-1 or (and) inhibition of protein kinase C and calcium/calmodulin pathway. Further investigations are underway to determine the possible mechanism(s) of this effect.

References


