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The effect of cyclosporine on the development and expression of cannabinoid tolerance in mice

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Abstract

Cyclosporine, beside its immunosuppressive action, has several effects on different neuronal functions, such as modulation of neurotransmitter release, the inhibition of nitric oxide synthesis and release, the reduction of cAMP production and inhibition of morphine-induced tolerance. In the present study, the effect of cyclosporine on the expression and development of tolerance to WIN 55,212-2, a cannabinoid receptor agonist, was studied. Intra peritoneal (i.p.) injection of WIN 55,212-2 (2–6 mg/kg) induced time-dependent and dose-dependent analgesia and catalepsy in mice. Administration of cyclosporine (20 mg/kg i.p.), 30 min before WIN 55,212-2 (6 mg/kg i.p.), did not change the analgesic and cataleptic effects of WIN 55,212-2. When WIN 55,212-2 (6 mg/kg i.p.) was injected once a day, animals became completely tolerant to the analgesic and cataleptic effects within five and nine days respectively. Cyclosporine (20 mg/kg i.p.) injected once daily, 30 min before WIN 55,212-2, attenuated the development of tolerance to the analgesic and cataleptic effects of WIN 55,212-2 but did not affect the expression of tolerance. Since cyclosporine given chronically by itself did not alter the analgesia and catalepsy induced by acute administration of WIN 55,212-2, our findings suggest cyclosporine may act with some selectivity on the mechanisms involved in development of cannabinoid tolerance.

Keywords: Cyclosporine; Cannabinoid; Tolerance; Mice

1. Introduction

Cannabinoids are a type of psychoactive compounds with many pharmacological properties similar to opioids. These properties include analgesia (Bloom and Dewy, 1978; Bhargava and Matwyshyn, 1980), hypothermia (Bhargava, 1980), sedation, hypotension and inhibition of both intestinal motility and locomotor activity (Holtzman et al., 1969). With chronic administration, tolerance develops to the analgesia and hypothermia (Pertwee, 1988) and catalepsy (Spina et al., 1998).

Cyclosporine (cyclosporin A) is a powerful suppressor of the immune system, widely used to prevent rejection of transplanted organ. The immunosuppressive effect of cyclosporine is due to binding to protein receptors, immunophilins (Handschumacher et al., 1984; Schreiber and Crabtree, 1992). 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 influences, regulation of intracellular calcium release and nitric oxide synthetase (NOS) (Steiner et al., 1996; Synder et al., 1998; Trajkovic et al., 1999). In the nervous system, cyclosporine reduces the catalytic activity of neuronal nitric oxide synthase (nNOS) through the inhibition of calcineurin-mediated dephosphrylation of nNOS, causing inhibition of NO release (Sharkey and Butcher, 1994; Rao et al., 1996; Sabatini et al., 1997). It has been shown that NOS inhibitors, such as L-NAME reduce tolerance to cannabinoid (Throat and Bhargava, 1994; Spina et al., 1998).

Further support for the effect of cyclosporine on cannabinoid tolerance is provided by our report that cyclosporine can attenuate morphine tolerance in mice (Homayoun et al., 2002) and guinea pig ileum model (Ejtemaei Mehr et al., 2003). 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 tolerance.

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Cyclosporine also reduces the adenylyl cyclase activity in some cell lines (Ockenfels et al., 1996a,b) and many studies have shown chronic cannabinoid treatment increases cAMP levels (Dill and Howlett, 1988; Fan et al., 1996; Rhee et al., 2000; Rubino et al., 2000).

To investigate the effect of cyclosporine on tolerance to cannabinoid, we choose analgesic and cataleptic effects of cannabinoid for assessment of tolerance. We examined the effect of cyclosporine on analgesia and catalepsy induced by acute and chronic exposure of WIN 55,212-2 (A cannabinoid receptor agonist).

2. Materials and methods

2.1. Animals

Male NMRI mice (Pasteur Institute of Iran) weighing 25–35 g at the time of the acute experiments or at the beginning of the chronic experiments were used. Animals were housed in a temperature-controlled room (24±1 °C) on a 12-h light/dark cycle with free access to food and water for at least four days before experiments. All experiments were carried out in the same room between 10:00 AM to 15:00 PM to minimize diurnal variations. Separate groups of animals were used for each test. All animal experiments were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 85-23, revised 1985).

2.2. Chemicals

A cannabinoid receptor agonist *R*(+)-[2,3-Dihydro-5-methyl-3[(morpholinyl) methyl] pyrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl) methanone mesylate (WIN 55,212-2) was purchased from Tocris Cookson (Bristol, UK). Cyclosporine from Sandoz Pharma (Basel, Switzerland). WIN 55,212-2 and cyclosporine were dissolved in 10% dimethylsulphoxide (DMSO) (V/V) in saline with 2–3 drops of emulphore.

2.3. Treatments

2.3.1. Acute treatment

In first experiments mice were injected i.p. with either vehicle or WIN 55,212-2 to assess the time-dependent and dose-dependent curves of the analgesic and cataleptic effects. In a second series of experiments, the effect of cyclosporine pretreatment (20 mg/kg i.p.) on analgesia and catalepsy induced by a single injection of WIN 55,212-2 (6 mg/kg i.p.) was studied. The mice were divided into four groups at random and received: vehicle+vehicle, cyclosporine+vehicle, vehicle+WIN 55,212-2, cyclosporine+WIN 55,212-2.

Vehicle or cyclosporine was injected i.p. 30 min before vehicle or WIN 55,212-2. Analgesia was measured 15 min and catalepsy was measured 30 min after vehicle or WIN 55,212-2 injection.

2.3.2. Chronic treatment

The effect of cyclosporine on the development and expression of tolerance to the analgesic and cataleptic effect of WIN 55,212-2 was studied by WIN 55,212-2 injection once a day for different days, depending on the effects considered (5 days for analgesia and 9 days for catalepsy). Mice allocated to four groups at random were treated with vehicle or cyclosporine (20 mg/kg i.p.) 30 min before vehicle or WIN 55,212-2 (6 mg/kg i.p.). In development study, cyclosporine was injected everyday but in expression study, it was injected only on test day (5th day for analgesia and 9th day for catalepsy). In the second series of experiments, the effect of chronic cyclosporine treatment (5 days for analgesia and 9 days for catalepsy) on the analgesia and catalepsy induced by single dose of WIN 55,212-2 was examined.

2.3.3. Analgesia

Antinociception was evaluated by the radiant heat tail flick test (D'Amour and Smith, 1941). Animals were restricted by a restrainer with their tail positioned in apparatus (Type 812, Hugo Sachs Electronics, Germany) for radiant heat stimulation on the dorsal surface of the tail. Tail-flick latency (TFL) was defined as the time interval between the application of standardized beam focused on the tail and the abrupt removal of the tail from nociceptive stimuli. The heat source was set so that baseline latencies were generally between 3 and 4 s. Cut-off time was set at 10 seconds. Antinociception was quantified as the percentage of the maximal effect (%MPE) using the following formula: %MPE=[(test latency−control latency)/(cut-off time−control latency)]×100.

2.3.4. Catalepsy

The cataleptic response was determined by using a slight modification of the ring test described by Pertwee (1972). Each mouse was placed on a ring (5.5 cm in diameter) which was attached to a stand and raised to a height of 16 cm. Mice were allowed to stay for a period of 5 min. During the test period, the sum of all times during which the mouse was motionless

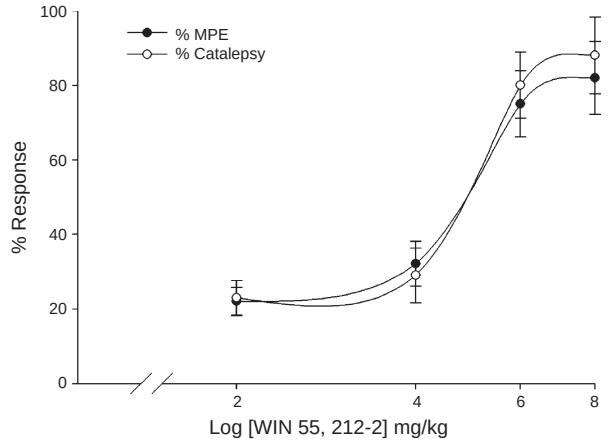

Fig. 1. The dose-dependent curve of analgesic and cataleptic effect of WIN 55,212-2 (6 mg/kg i.p.). Each point represents the mean±S.E.M. of measurement of eight mice. %MPE=[(test latency−control latency)/(cut-off time−control latency)]×100.

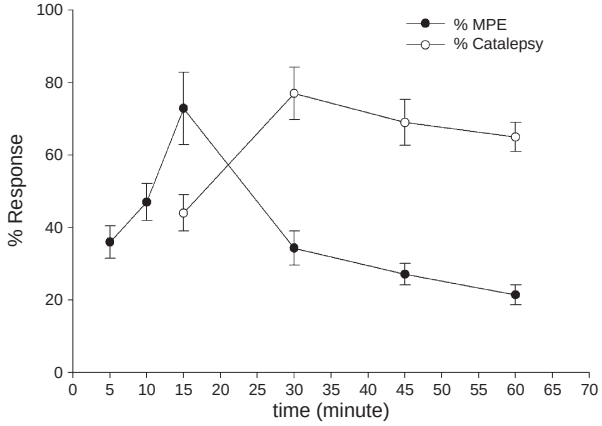

Fig. 2. The time-dependent curve of analgesic and cataleptic effect of WIN 55,212-2 (i.p.). Each point represents the mean±S.E.M. of measurements of eight mice.

(except for respiratory movements) was measured. This value was divided by 300 s and multiplied by 100 to obtain the catalepsy percentage.

2.3.5. Statistical analysis

The results were expressed as the mean±S.E.M. The data were analyzed using Graphpad Prism data analysis program (Graphpad software San Diego, CA, USA). 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. Differences were considered significant at $p<0.05$.

3. Results

3.1. Acute treatment

The dose-dependent curve of WIN 55,212-2-induced analgesia and catalepsy was initially obtained to select the dose of WIN 55,212-2 (6 mg/kg i.p.) producing about 70–80% analgesic or cataleptic effect for experiments. Analgesia and catalepsy were linearly related to the administrated dose in the range between 2 and 6 mg/kg (Fig. 1).

The time-dependent curve of WIN 55,212-2-induced analgesia and catalepsy was examined. Analgesic effect peaked 15 min and catalepsy 30 min after WIN 55,212-2 (6 mg/kg) injection (Fig. 2).

To study the influence of cyclosporine (20 mg/kg i.p.) on WIN 55,212-2 analgesia and catalepsy, it was injected 30 min

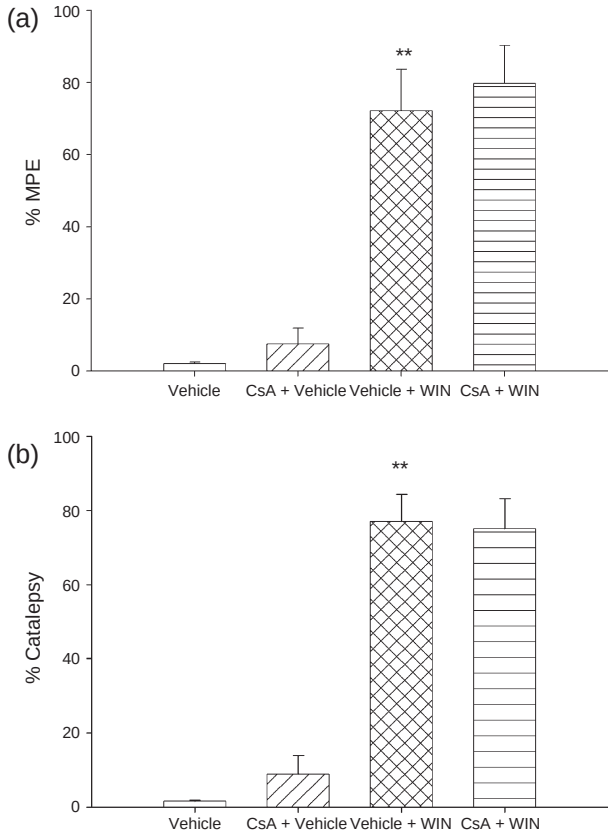

Fig. 3. Effect of cyclosporine (CsA) (20 mg/kg i.p.) on analgesia (a) and catalepsy (b) induced by WIN 55,212-2 (6 mg/kg i.p.). Cyclosporine (20 mg/kg i.p.) was administrated 30 min before WIN 55,212-2 injection. Analgesia and catalepsy were measured after 15 and 30 min in different groups respectively. $^{**}P<0.001$ versus vehicle. Each bar represents the mean±S.E.M. of measurements of eight mice.

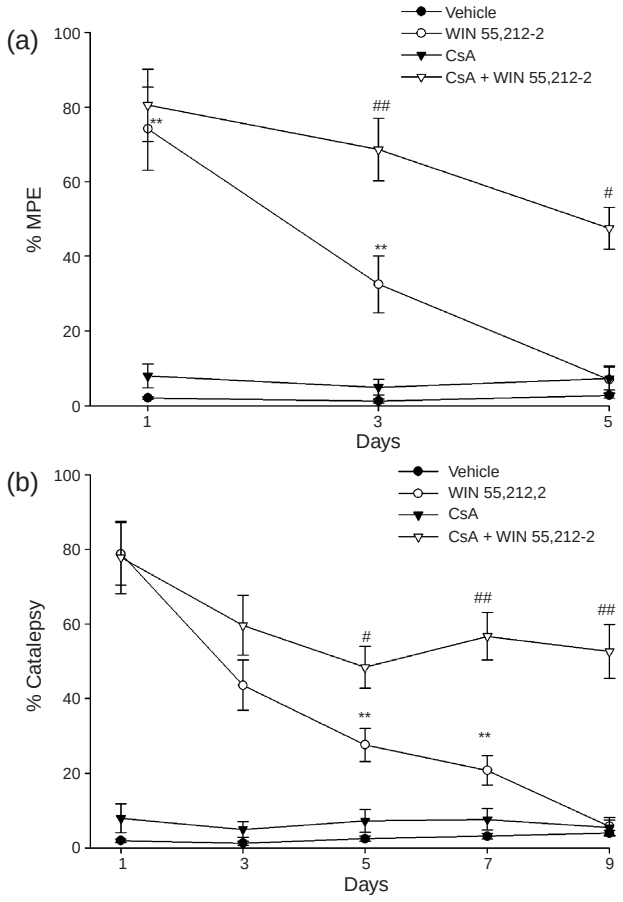

Fig. 4. Effect of cyclosporine on the development of tolerance to the analgesia (a) and catalepsy (b) of WIN 55,212-2 (6 mg/kg i.p.). CsA (20 mg/kg i.p.) was administrated 30 minutes before WIN 55,212-2 injection. Analgesia and catalepsy were measured after 15 and 30 min in different groups, respectively. $^{**}P<0.001$ versus vehicle; $^{\#\#}P<0.001$ versus vehicle+WIN 55,212-2. Each point represents the mean±S.E.M. of measurements of eight mice.

before the cannabinoid and the response was measured at the peak time. Cyclosporine was ineffective and did not affect the analgesic (Fig. 3a) and cataleptic responses (Fig. 3b).

3.2. Chronic treatment

The effect of cyclosporine on development of tolerance to the analgesic effect of WIN 55,212-2 is shown in Fig. 4a. Repeated administration of WIN 55,212-2 (6 mg/kg i.p.) elicited a progressively lower response and after five days, %MPE decreased from 74.2±11.1 to 6.9±3.7. The concurrent administration of cyclosporine significantly attenuated the development of WIN 55,212-2 tolerance, so that after five days of daily WIN 55,212-2, the percentage of MPE change for the cyclosporine plus WIN 55,212-2 group was 47.5±5.6 compared with 6.9±3.7 for vehicle plus WIN 55,212-2 group, $F(3,28)=32{,}131$ $p<0.001$(Fig. 4a).

Continued dosing of WIN 55,212-2 also resulted in the development of tolerance to the cataleptic effect, which was reduced by coadministration of cyclosporine. With WIN 55,212-2 alone, the catalepsy declined from 78.8±8.4 to 5.8±2.4 over nine days; while with cyclosporine, it never fell below 52.6±7.2, $F(3,28)=36.2$ $p<0.001$ (Fig. 4b).

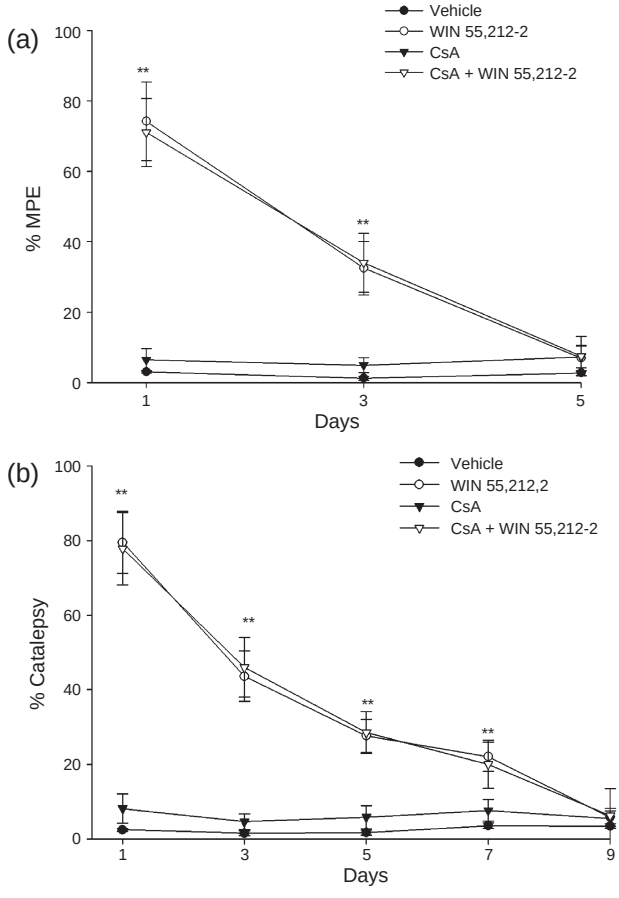

Fig. 5. Effect of cyclosporine on the expression of tolerance to the analgesia (a) and catalepsy (b) of WIN 55,212-2 (6 mg/kg i.p.). CsA (20 mg/kg i.p.) was administrated 30 min before WIN 55,212-2 injection. Analgesia and catalepsy were measured after 15 and 30 min in different groups, respectively. $^{**}P<0.001$ versus vehicle. Each point represents the mean±S.E.M. of measurements of eight mice.

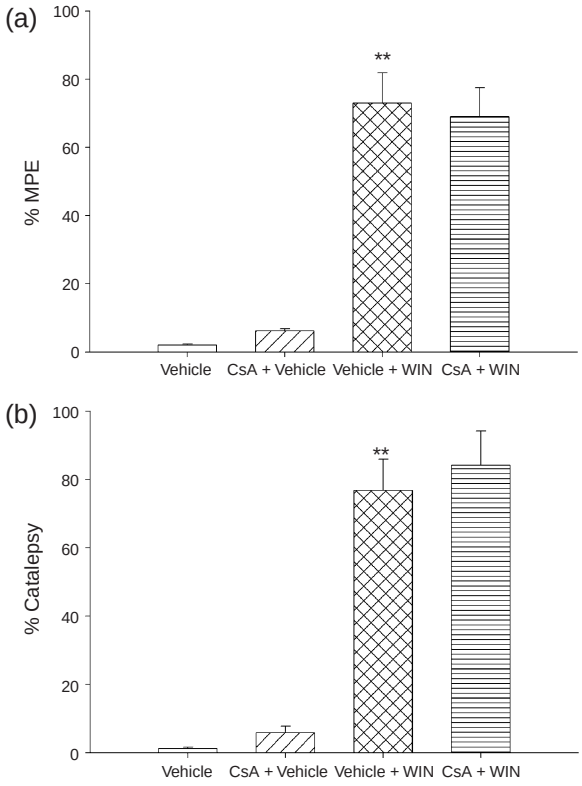

Fig. 6. Effect of repeated injection of cyclosporine (CsA) (20 mg/kg i.p.) on analgesia (a) and catalepsy (b) induced by WIN 55,212-2 (6 mg/kg i.p.) 30 min after CsA injection on 5th day for analgesia and on 9th for catalepsy. Analgesia and catalepsy were measured after 15 and 60 min in different groups, respectively. A double vehicle treatment was used for control. $^{**}P<0.001$ versus vehicle. Each bar represents the mean±S.E.M. of measurements of eight mice.

Administration of cyclosporine in expression phase of tolerance to cannabinoid did not change the analgesic or cataleptic responses and could not inhibit the expression of tolerance to WIN 55,212-2 (Fig. 5).

Chronic treatment with cyclosporine did not alter either the analgesic response to a single injection of WIN 55,212-2 on 5th day (Fig. 6a), or the cataleptic response to WIN 55,212-2 given on 9th day of cyclosporine administration (Fig. 6b).

4. Discussion

Our data show that systemic administration of cyclosporine during the induction phase attenuates the development of tolerance to WIN 55,212-2-induced analgesia and catalepsy but does not affect the expression of tolerance.

Cyclosporine following either acute or chronic treatment did not affect the analgesia or catalepsy induced by a single injection of WIN 55,212-2. The observation that cyclosporine blocks the development of tolerance to analgesic and cataleptic effects of WIN 55,212-2 without altering its acute actions in mice explains the effect of cyclosporine with some selectivity on the mechanisms involved in development of cannabinoid tolerance.

The results show that the different time course of development of tolerance to the analgesic and cataleptic effects induced by WIN 55,212-2 (5 and 9 days, respectively). This pattern is may be related to the different sites and pathways that they mediate these effects. The effect of cannabinoid in suppression of tail flick responses elicited from the dorsal periaqueductal gray (PAG) in the midbrain, the rostral ventrolateral medulla (RVM) and the noradrenergic nucleus A_5 in the medulla (Walker and Huang, 2002) but the cataleptic effect of cannabinoid was mediated through CB_1 receptors were found in basal ganglia (Martin, 1986). In addition, NO is involved in the development of tolerance to the cataleptic effect induced by WIN 55,212-2 but does not mediate tolerance to the analgesic effect of cannabinoid (Spina et al., 1998).

Recently, we have reported that immunophilin ligands (Cyclosporine and FK 506) can attenuate the development and expression of tolerance to morphine (Homayoun et al., 2002, 2003; Ejtemaei Mehr et al., 2003). It is obvious that cannabinoids and opioids have similar pharmacological properties, common signaling mechanism and cross-tolerance (Shapira et al., 2003), But in the present study cyclosporine could not decrease the expression of tolerance to WIN 55,212-2. The fact that cyclosporine can attenuate the expression of morphine-induced tolerance but can not decease the expression of tolerance to WIN 55,212-2, is related to the acute effect of cyclosporine in enhancing of morphine antinociception (Homayoun et al., 2002), this effect increases the antinociception score during the expression phase of morphine tolerance. Nevertheless, cyclosporine did not change the analgesic and cataleptic effect of WIN 55,212-2 (Fig. 3). In the other hand, this result is may be due to the differences between opioid and cannabinoid system.

It has been shown that, in the nervous system, cyclosporine reduces the catalytic activity of neuronal nitric oxide synthase and subsequently causes the inhibition of nitric oxide release (Dawson et al., 1993; Sharkey and Butcher, 1994; Rao et al., 1996). This mechanism has been implicated in some of the important functions of cyclosporine in the nervous system (Sabatini et al., 1997; Synder et al., 1998; Ruiz et al., 2000). Inhibitory effect of nitric oxide synthase inhibitors on the development of WIN 55,212-2-induced tolerance has been previously reported (Spina et al., 1998) and it is possible that cyclosporine decrease the development of cannabinoid tolerance through L-arginine/nitric oxide pathway.

It has been reported that cyclosporine reduces the cAMP level in some cell lines (Ockenfels et al., 1996a,b) and many studies have shown chronic cannabinoid treatment increases cAMP levels (Dill and Howlett, 1988; Fan et al., 1996; Rhee et al., 2000; Rubino et al., 2000). Therefore, another possible mechanism of cyclosporine in modulation of cannabinoid tolerance is the reduction of cAMP formation.

In summary, the present findings clearly demonstrated that systemic administration of cyclosporine could attenuate the development of tolerance to WIN 55,212-2 in mice, without any effect on expression of WIN 55,212-2 tolerance. More studies are needed to find the underlying mechanism(s) of this effect.

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