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Role of GLT-1 transporter activation in prevention of cannabinoid tolerance by the beta-lactam antibiotic, ceftriaxone, in mice

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ABSTRACT

Recently, it has been indicated that beta lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. Furthermore, these antibiotics have been shown to prevent the development of tolerance and dependence to opioids. Since cannabinoid tolerance is known to be similar to opioids, our purpose was to examine the effect of ceftriaxone on the development of tolerance to WIN 55,212-2, a cannabinoid agonist. The tail flick test, a rectal thermometer, and the ring test were used for evaluating the degree of tolerance to the analgesic, hypothermic, and cataleptic effects of WIN 55,212-2, respectively. Within one week, animals became completely tolerant to analgesic, hypothermic and cataleptic effects of WIN 55,212-2 (6 mg/kg). Ceftriaxone, with its higher doses (100–200 mg/kg), attenuated the development of tolerance to the analgesic and hypothermic effects of WIN 55,212-2, but had no effect on its cataleptic action. Dihydrokainic acid (10 mg/kg), a GLT-1 transporter inhibitor, prevented this effect of ceftriaxone. Our results suggest that repeated treatment with ceftriaxone prevents the development of tolerance to the analgesic and hypothermic effects of cannabinoids, and GLT-1 activation appears to play a key role in this preventive effect of beta-lactam antibiotics.

1. Introduction

Glutamate is the primary excitatory amino acid neurotransmitter in the central nervous system, and extracellular glutamate homeostasis is principally regulated by glutamate transporter system. Five types of excitatory amino acid transporters have been identified: EAAT1 (GLAST), EAAT2 (Glutamate transporter-1, GLT-1), EAAT3, EAAT4, and EAAT5 (Seal and Amara, 1999; Danbolt, 2001). GLT-1 transporters appear to play the key role in terminating synaptic transmission of glutamate, since they mediate 90% of glutamate uptake (Danbolt, 2001). Beta-lactam antibiotics, such as ceftriaxone, have been shown to offer neuroprotection by stimulating GLT-1 expression (Rothstein et al., 2005). GLT-1 dysfunction contributes to amyotrophic lateral sclerosis, Parkinson's disease, seizure, and opioid tolerance and dependence (Rao et al., 2001; Rawls et al., 2008, 2010): moreover, recent investigations indicate an antinociceptive role for GLT-1 activation in visceral and nerve injury-induced neuropathic pain states (Lin et al., 2009; Hu et al., 2010; Yan et al., 2009).

Opioids and cannabinoids are two distinct drug classes sharing many pharmacological properties, such as analgesia, hypothermia, sedation, and hypoactivity (Fuentes et al., 1999; Pertwee, 2001; Walker and Huang, 2002; Banafshe et al., 2005). Similarly, long-term use of both classes of these drugs is associated with the development of tolerance and physical dependence (Fuentes et al., 1999; Pertwee, 2001; Walker and Huang, 2002; Shapira et al., 2003; Banafshe et al., 2005; Gunduz et al., 2010). Distinct G-protein coupled opioid and cannabinoid receptors mediate these effects. Recently, analgesic and hypothermic tolerance to opioids have been shown to be prevented by ceftriaxone, a GLT-1 transporter activator (Wang et al., 2008; Rawls et al., 2008, 2010). Considering the similarities between opioids and cannabinoids, a similar beta-lactam effect on cannabinoid tolerance might also be expected.

The aim of our study is, therefore, to observe the effect of ceftriaxone, a beta lactam antibiotic, on the development of tolerance to the analgesic, hypothermic and cataleptic effects of WIN 55,212-2, a cannabinoid agonist.

2. Materials and methods

2.1. Animals

Male Balb-c albino mice (Center of the Laboratory Animals, Trakya University), weighing 20–30 g at the beginning of the experiments, were used. Animals were housed in groups of ten in a quiet room, and water and food were provided ad libitum. This study was conducted according to the guidelines of the Ethical Committee of the International Association for the Study of Pain (Zimmermann, 1983), and the local "Animal Care Ethics Committee" approved the experimental protocols.

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2.2. Tail flick test

Tail-flick test (Commat, Ankara, Turkey) was used to assess the antinociceptive response. Briefly, a beam of light was focused on the dorsal surface of the mouse tail and the time until the tail flicked was measured. At the beginning of the experiments, the light intensity in the apparatus was set such that the baseline tail-withdrawal latencies were approximately 2–4 s in all mice. A cut-off time of 10 s was adopted in order to minimize tissue damage. Test latencies were converted to the percentage of the maximal possible effect (%MPE) according to the following formula: %MPE = [(postdrug latency − baseline latency) / (cut-off time − baseline latency)] × 100.

2.3. Measurement of rectal temperature

The temperature was measured to the nearest 0.1 °C by an Ellab thermometer. This was done by inserting the probe (2 mm in diameter) 2.5 cm into the rectum of mice. The probe was left in place until steady readings were obtained (20–25 s). Baseline readings (average of two trials) were taken for each animal immediately before the injections of WIN 55,212-2. Animals were allowed to acclimate for 1 h prior to baseline readings. Thirty min after WIN 55,212-2 injections, body temperature was measured in duplicates and mean was drawn for further calculations. Data were expressed as the mean ± SEM. of the change in body temperature from baseline.

2.4. Ring test

The cataleptic response was measured by the modified 'ring test' (Fox et al., 2001), originally described by Pertwee (1972). Mice were placed by their forepaws over a fixed wire ring (5.5 cm in diameter), which allowed their hindpaws to just touch the tabletop. Latencies for the mouse to move off the ring were measured, the cut-off time being 30 s. Test latencies were converted to the percentage of the maximal possible effect (%MPE) according to the following formula: %MPE = [(postdrug latency − baseline latency) / (cut-off time − baseline latency)] × 100.

2.5. Experimental protocol

Tolerance to analgesic, hypothermic and cataleptic effects of WIN 55,212-2 was induced by i.p. injections of the drug (6 mg/kg) once daily for 7 days (Spina et al., 1998). The analgesia, hypothermia and catalepsy tests were conducted immediately before and 30 min after the morning injections of WIN 55,212-2, and averaged from two trials separated by 2 min intervals. In order to observe the effect of beta-lactam antibiotic treatment on the development of cannabinoid tolerance, ceftriaxone (50–200 mg/kg) was administered 15 min before WIN 55,212-2 for 7 days. In another group, dihydrokainic acid (10 mg/kg), a GLT-1 blocker, was co-administered with ceftriaxone for 7 days, to determine if GLT-1 activation mediate the beta-lactam effect.

2.6. Drugs

WIN 55,212-2 and DMSO were purchased from Sigma Chemical Co., while dihydrokainic acid was obtained from Tocris. Ceftriaxone (Rocephin, Roche) was diluted from commercial preparations. Ceftriaxone and dihydrokainic acid were dissolved in saline, and WIN 55,212-2 was prepared in 20% DMSO/1% ethanol/1% Tween-80/78% saline. All chemicals were administered i.p. in a volume of 0.1 ml/10 g body weight.

2.7. Statistical analysis

All data are expressed as mean ± SEM. Statistical comparisons among groups were carried out using two-way analysis of variance (ANOVA) with repeated measures on time, followed by Bonferroni-*t* test. Values of $p<0.05$ were considered to be significant.

3. Results

3.1. Effects of repeated ceftriaxone treatment on cannabinoid tolerance

Repeated administration of 6 mg/kg of WIN 55,212-2 once daily elicited a progressive decrease in the analgesia test; treatment effect [F (1, 10) = 172.13, $p<0.0001$], day effect [F (6, 60) = 16.29, $p<0.0001$], and treatment × day interactions [F (6, 60) = 18.38, $p<0.0001$] were significant (Fig. 1A). Tolerance also developed to the hypothermic and cataleptic effects of chronic WIN 55,212-2. For the hypothermic tolerance, two-way ANOVA revealed a significant treatment effect [F (1, 10) = 115.73, $p<0.0001$], day effect [F (6, 60) = 12.49, $p<0.0001$], and treatment × day interaction [F (6, 60) = 6.53, $p<0.0001$] (Fig. 1B). Treatment effect [F (1, 10) = 53.90, $p<0.0001$], day effect [F (6, 60) = 12.94, $p<0.0001$], and treatment × day interaction [F (6, 60) = 8.97, $p<0.0001$] were also significant in the development of cataleptic tolerance to WIN 55,212-2 (Fig. 1C). Tolerance to

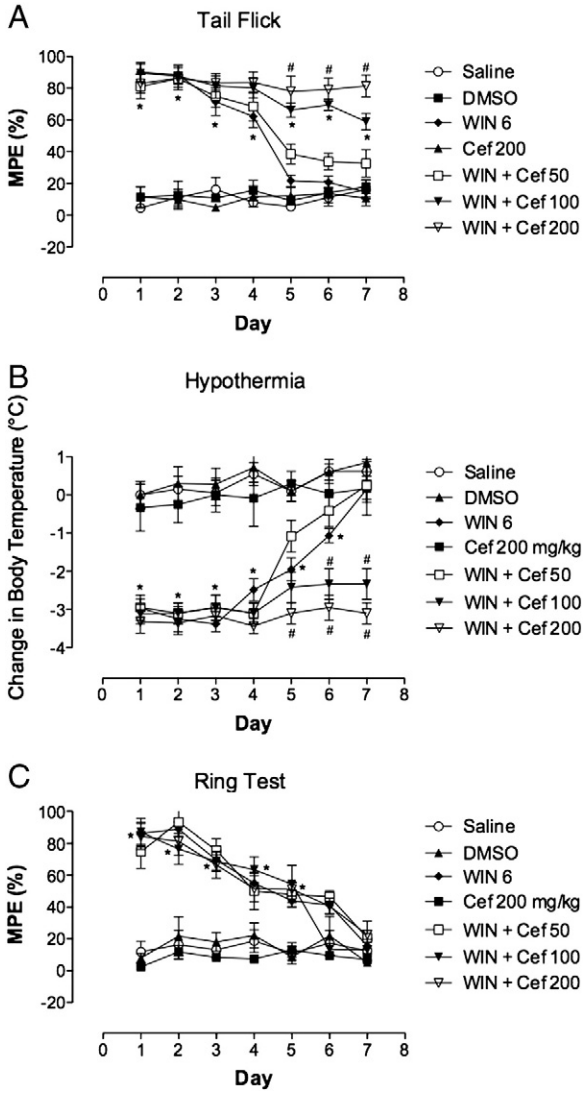

Fig. 1. Effects of repeated administration of ceftriaxone (50–200 mg/kg; n = 6–7 for each dose) on the development of tolerance to the analgesic (A), hypothermic (B) and cataleptic (C) effects of WIN 55,212-2 (6 mg/kg). (*$p<0.05$, compared to corresponding values in the control group; #$p<0.05$, compared to corresponding values in the WIN-tolerant group).

the analgesic effect of WIN 55,212-2 developed by day 5 of testing; on the other hand, hypothermic and cataleptic tolerance developed totally by day 7 (Fig. 1A, B and C). When administered alone, neither DMSO (control for WIN 55,212-2) nor ceftriaxone (200 mg/kg) altered the baseline nociceptive, hypothermic, and cataleptic responses (Fig. 1A, B and C).

Repeated ceftriaxone treatment prevented tolerance to the analgesic effect of WIN 55,212-2, with a significant treatment effect [$F(3, 20) = 27.41$, $p<0.0001$], day effect [$F(6, 120) = 30.55$, $p<0.0001$], and interaction [$F(18, 120) = 4.64$, $p<0.0001$] (Fig. 1A). Repeated administration of the beta lactam antibiotic also resulted in the prevention of tolerance to the hypothermic effect of WIN 55,212-2; treatment effect [$F(3, 20) = 23.21$, $p<0.0001$], day effect [$F(6, 120) = 26.73$, $p<0.0001$], and treatment × day interactions [$F(18, 120) = 4.88$, $p<0.0001$] were significant (Fig. 1B). Ceftriaxone did not affect the development of tolerance to the cataleptic actions of WIN 55,212-2 (Fig. 1C). In general, mice treated with 200 mg/kg of ceftriaxone displayed greater efficacy than 50 and 100 mg/kg doses (Fig. 1A, B and C).

3.2. Prevention of inhibitory effect of ceftriaxone on cannabinoid tolerance by GLT-1 transporter inhibition

Ceftriaxone (200 mg/kg)-induced inhibition of tolerance to WIN 55,212-2-evoked analgesia and hypothermia was prevented, when co-administered with dihydrokainic acid, at a dose that produced no effect on its own (10 mg/kg, i.p., Fig. 2A, B and C). Two-way ANOVA revealed a significant treatment effect [$F(1, 10) = 24.85$, $p = 0.0005$], day effect [$F(6, 60) = 7.63$, $p<0.0001$], and treatment × day interaction [$F(6, 60) = 5.41$, $p = 0.0002$] in the tail-flick test. In prevention of hypothermic tolerance by dihydrokainic acid, two-way ANOVA also revealed a significant treatment effect [$F(1, 10) = 16.72$, $p = 0.0022$], day effect [$F(6, 60) = 10.24$, $p<0.0001$], and treatment × day interaction [$F(6, 60) = 6.70$, $p<0.0001$]. In mice that are not treated with ceftriaxone, dihydrokainic acid did not influence WIN 55,212-2-induced analgesic and hypothermic tolerance (Fig. 2A, B and C).

4. Discussion

A major limitation to the chronic usage of opioids is the development of tolerance to their analgesic effects, leading to dose escalation and augmentation of adverse effects (Martin and Eisenach, 2001). Tolerance limits the utility of cannabinoids as well as opioids during repeated treatment (Fuentes et al., 1999; Pertwee, 2001; Walker and Huang, 2002; Banafshe et al., 2005). Although cannabinoid tolerance seems to be similar to opioids, the precise mechanisms mediating the development of tolerance to these potent analgesics are not totally identified. In the present study, repeated administration of WIN 55,212-2, a cannabinoid agonist, elicited a progressive decrease in the analgesic, hypothermic and cataleptic responses. Ceftriaxone inhibited the development of tolerance to the analgesic and hypothermic actions but not to cataleptic action of WIN 55,212-2. Dihydrokainic acid, a GLT-1 transporter inhibitor, prevented this inhibitory effects of ceftriaxone, indicating that ceftriaxone efficacy against WIN 55,212-2 tolerance is dependent on GLT-1 activation.

Despite intensive investigation, the precise mechanisms mediating opioid tolerance are only partially understood (Bhargava, 1994). Cannabinoid analgesic tolerance also appears to be similar to opioids (Fuentes et al., 1999; Pertwee, 2001; Walker and Huang, 2002; Shapira et al., 2003; Banafshe et al., 2005; Ulugol, 2009); agonist-induced down-regulation and intracellular trafficking of cannabinoid receptors seem to be involved in cannabinoid analgesic tolerance, but not much is known about these molecular mechanisms. Taking into account the cross-tolerance and dependence between opioids and cannabinoids, and recent reports indicating that beta-lactam antibiotics prevent the development of opioid analgesic and hypothermic

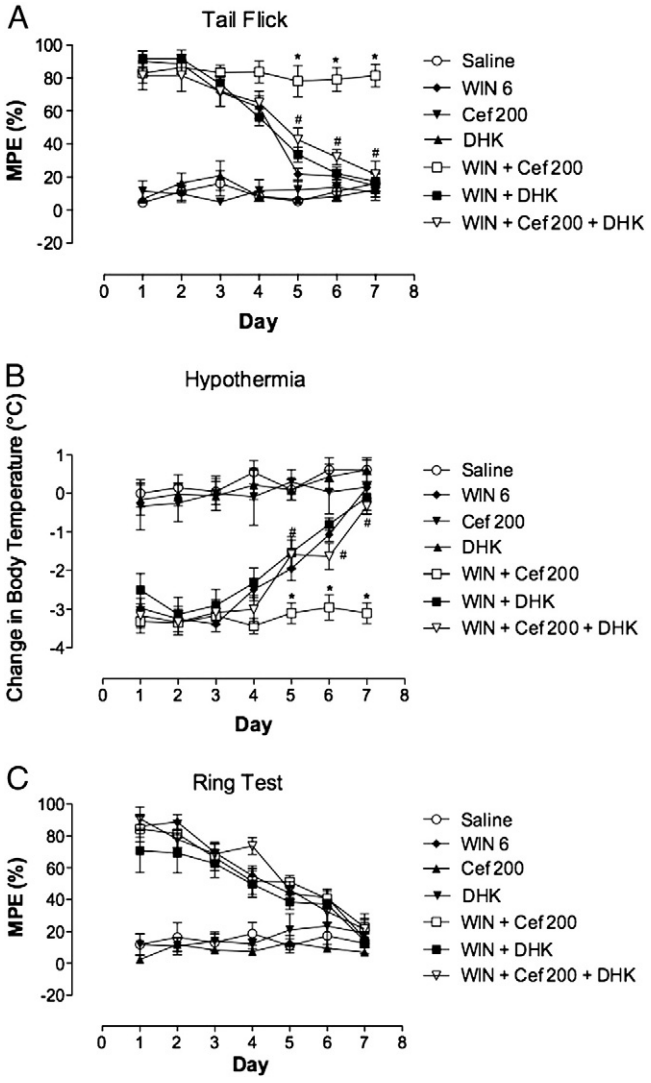

Fig. 2. Effects of the GLT-1 transporter inhibitor, dihydrokainic acid (10 mg/kg), on the ceftriaxone (200 mg/kg) prevention of the development of tolerance to the analgesic (A), hypothermic (B) and cataleptic (C) effects of WIN 55,212-2 (6 mg/kg). ($^{*}p<0.05$, compared to corresponding values in the WIN-tolerant group; $^{\#}p<0.05$, compared to corresponding values in the WIN + ceftriaxone group).

tolerance by increasing GLT-1 transporter function (Wang et al., 2008; Rawls et al., 2008, 2010), it can be speculated that GLT-1 activation might also be involved in cannabinoid tolerance. Our findings are in line with this hypothesis, since we observed that ceftriaxone inhibited the development of analgesic and hypothermic tolerance to the cannabinoid agonist, WIN 55,212-2, and dihydrokainic acid, a GLT-1 transporter inhibitor, prevented these inhibitory effects of the beta-lactam antibiotic.

The mechanism by which GLT-1 transporter activation affects cannabinoid tolerance is unclear. Glutamate receptor antagonists have been shown to prevent WIN 55,212-2-induced antinociception with the participation of metabotropic and NMDA glutamate receptors (Palazzo et al., 2001). Suppression of glutamate-dependent component of tolerance to antinociceptive and hypothermic effects of opioids has been proposed as the most probable explanation for prevention of these effects by ceftriaxone (Rawls et al., 2008, 2010). Thus, in cannabinoid-induced analgesic and hypothermic tolerance, ceftriaxone might also increased GLT-1 transporter activity and enhanced glutamate activity, which in turn prevented the increase of extracellular glutamate levels during repeated WIN 55,212-2 exposure. On the contrary, MK-801, a competitive NMDA receptor

antagonist, antagonized the analgesic but not the hypothermic effects of delta 9-tetrahydrocannabinol, whereas, pretreatment with MK-801 failed to affect the development of tolerance to the analgesic and hypothermic actions of delta 9-tetrahydrocannabinol (Thorat and Bhargava, 1994). More studies are, therefore, needed to further clarify the mechanism by which GLT-1 transporter activation prevents the development of cannabinoid analgesic and hypothermic tolerance.

In our study, ceftriaxone had no effect on the cataleptic action of the cannabinoid drug. These results indicate that tolerance to the cataleptic effect of WIN 55,212-2 might require mechanisms other than reduced GLT-1 transporter activity. This deduction seems reasonable, because different sites and pathways of the central nervous system mediate different effects of cannabinoids. For example, the cataleptic effect of cannabinoids was mentioned to be mediated through CB1 receptors in basal ganglia, whereas the analgesic and the hypothermic effects are mediated through different regions, such as medulla and hypothalamus (Martin, 1986; Herkenham et al., 1990). Similarly, NO mediate tolerance to the cataleptic but not to the analgesic effect of WIN 55,212-2 (Spina et al., 1998). These reports, in accordance with our findings, indicate a different mechanism for the cataleptic action of cannabinoids.

In summary, our results demonstrate that ceftriaxone, possibly by increasing glutamate uptake, prevents the development of tolerance to the analgesic and hypothermic effects of WIN 55,212-2. GLT-1 activation by beta lactam antibiotics appears to be a promising approach in preventing the development of tolerance to cannabinoids. The mechanism by which GLT-1 transporter activation prevents cannabinoid tolerance is not clear, but it seems that increased cellular glutamate uptake reduces glutamate levels and decreases activation of glutamate receptors; however, further studies, such as data of the effect of ceftriaxone on the acute analgesic and hypothermic effects of WIN 55,212-2, are required to delineate the entire mechanism of action of ceftriaxone. Impairing the development of tolerance to cannabinoids by beta lactam antibiotics may provide the use of cannabinoids as alternative therapeutics in chronic pain states, by increasing their analgesic efficacy and reducing their undesirable effects.

References

Banafshe HR, Ghazi-Khansari M, Dehpour AR. The effect of cyclosporine on the development and expression of cannabinoid tolerance in mice. Pharmacol Biochem Behav 2005;82:658–63.

Bhargava HN. Diversity of agents that modify opioid tolerance, physical dependence, abstinence syndrome and self-administrative behaviour. Pharmacol Rev 1994;46: 293–324.

Danbolt NC. Glutamate uptake. Prog Neurobiol 2001;65:1-105.

Fox A, Kesingland A, Gentry C, McNair K, Patel S, Urban L, et al. The role of central and peripheral Cannabinoid$_1$ receptors in the antihyperalgesic activity of cannabinoids in a model of neuropathic pain. Pain 2001;92:91-100.

Fuentes JA, Ruiz-Gayo M, Manzanares J, Vela G, Reche I, Corchero J. Cannabinoids as potential new analgesics. Life Sci 1999;65:675–85.

Gunduz O, Karadag CH, Ulugol A. Modulatory role of the endogenous nitric oxide synthase inhibitor, asymmetric dimethylarginine (ADMA), in morphine tolerance and dependence in mice. J Neural Transm 2010;117:1027–32.

Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, De Costa BR, et al. Cannabinoid receptor localization in brain. Proc Natl Acad Sci U S A 1990;87: 1932–6.

Hu Y, Li W, Lu L, Cai J, Xian X, Zhang M, et al. An anti-nociceptive role for ceftriaxone in chronic neuropathic pain in rats. Pain 2010;148:284–301.

Lin Y, Tian G, Roman K, Handy C, Travers JB, Lin CG, et al. Increased glial glutamate transporter EAAT2 expression reduces visceral nociceptive response in mice. Am J Physiol Gastrointest Liver Physiol 2009;296:G129–34.

Martin BR. Cellular effects of cannabinoids. Pharmacol Rev 1986;38:45–74.

Martin TJ, Eisenach JC. Pharmacology of opioid and nonopioid analgesics in chronic pain states. J Pharmacol Exp Ther 2001;299:811–7.

Palazzo E, Marabese I, de Novellis V, Oliva P, Rossi F, Berrino L, et al. Metabotropic and NMDA glutamate receptors participate in the cannabinoid-induced antinociception. Neuropharmacology 2001;40:319–26.

Pertwee RG. The ring test: a quantitative method for assessing the cataleptic effect of cannabis in mice. Br J Pharmacol 1972;46:753–63.

Pertwee RG. Cannabinoid receptors and pain. Prog Neurobiol 2001;63:569–611.

Rao VL, Bowen KK, Dempsey RJ. Transient focal cerebral ischemia down-regulates glutamate transporters GLT-1 and EAAC1 expression in rat brain. Neurochem Res 2001;26:497–502.

Rawls SM, Robinson W, Patel S, Baron A. Beta-lactam antibiotic prevents tolerance to the hypothermic effect of a kappa opioid receptor agonist. Neuropharmacology 2008;55:865–70.

Rawls SM, Zielinski M, Patel H, Sacavage S, Baron DA, Patel D. Beta-lactam antibiotic reduces morphine analgesic tolerance in rats through GLT-1 transporter activation. Drug Alcohol Depend 2010;107:261–3.

Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DE, et al. Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. Nature 2005;433:73–7.

Seal RP, Amara SG. Excitatory amino acid transporters: a family in flux. Annu Rev Pharmacol Toxicol 1999;39:431–56.

Shapira M, Gafni M, Sarne Y. Long term interaction between opioid and cannabinoid agonists at the cellular level: cross-desensitization and down regulation. Brain Res 2003;960:190–200.

Spina E, Trovati A, Parolaro D, Giagnoni G. A role of nitric oxide in WIN 55,212-2 tolerance in mice. Eur J Pharmacol 1998;343:157–63.

Thorat SN, Bhargava HN. Effect of NMDA receptor blockade and NOS inhibition on the acute and chronic action of delta 9-tetrahydrocannabinol in mice. Brain Res 1994;667:77–82.

Ulugol A. Modulatory role of asymmetric dimethylarginine (ADMA) in cannabinoid tolerance and dependence. Med Hypotheses 2009;73:213–4.

Walker JM, Huang SM. Cannabinoid analgesia. Pharmacol Ther 2002;95:127–35.

Wang M, Dong HJ, Gong ZH. Effects of beta-lactam antibiotics on development of tolerance and dependence to morphine. Yao Xue Xue Bao 2008;43:1094–8.

Yan H, Li CM, Li YL, Gong ZH. Effect of glutamate transporter 1 on chronic constriction injury of sciatic nerve and morphine tolerance of rats. Yao Xue Xue Bao 2009;44: 581–5.

Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109–10.