Neuropsychopharmacology (2006) 31, 795–803
© 2006 Nature Publishing Group All rights reserved 0893-133X/06 $30.00
www.neuropsychopharmacology.org

Cannabidiol Reverses MK-801-Induced Disruption of Prepulse Inhibition in Mice

Leonora E Long*[,1], Daniel T Malone[1] and David A Taylor[1]

[1]*Department of Pharmaceutical Biology and Pharmacology, Victorian College of Pharmacy, Monash University, Victoria, Australia*

Cannabidiol, a nonpsychoactive constituent of the *Cannabis sativa* plant, has been reported to act as an agonist of the vanilloid 1 channel in the transient receptor potential family (TRPV1) and also to inhibit the hydrolysis and cellular uptake of the endogenous cannabinoid anandamide. Cannabidiol has also been reported to have potential as an antipsychotic. We investigated the effect of cannabidiol on sensorimotor gating deficits in mice induced by the noncompetitive NMDA receptor antagonist, MK-801. Sensorimotor gating is deficient in psychotic disorders such as schizophrenia and may be reliably measured by prepulse inhibition (PPI) of the startle response in rodents and humans. MK-801 (0.3–1 mg/kg i.p.) dose dependently disrupted PPI while cannabidiol (1–15 mg/kg i.p.), when administered with vehicle, had no effect on PPI. Cannabidiol (5 mg/kg i.p.) successfully reversed disruptions in PPI induced by MK-801 (1 mg/kg i.p.), as did the atypical antipsychotic clozapine (4 mg/kg i.p.). Pretreatment with capsazepine (20 mg/kg i.p.) prevented the reversal of MK-801-induced disruption of PPI by cannabidiol, providing preliminary evidence that TRPV1 receptors are involved in the reversal of MK-801-induced sensorimotor gating deficits by cannabidiol.
Neuropsychopharmacology (2006) **31,** 795–803. doi:10.1038/sj.npp.1300838; published online 27 July 2005

Keywords: cannabidiol; capsazepine; endocannabinoid; schizophrenia; sensorimotor gating; vanilloid receptor

INTRODUCTION

Cannabidiol is a nonpsychoactive constituent of the *Cannabis sativa* plant. The major psychoactive *Cannabis* constituent Δ^9-tetrahydrocannabinol (Δ^9-THC) activates at least two cannabinoid receptors, CB_1 and CB_2, which are coupled to G-proteins (Howlett *et al*, 2002). Unlike Δ^9-THC, cannabidiol has very weak affinity for CB_1 and CB_2 receptors (Bisogno *et al*, 2001) and does not alter neural uptake of amine neurotransmitters such as dopamine and 5-HT (Hershkowitz and Szechtman, 1979) or excite dopaminergic nerve cell firing (French *et al*, 1997). Cannabidiol acts as a full agonist *in vitro* at the transient receptor potential vanilloid receptor (TRPV1), with desensitization and maximal stimulation similar to the prototypical TRPV1 agonist capsaicin (Bisogno *et al*, 2001). Cannabidiol produces anti-inflammatory activity in rats mediated by TRPV1 but not by CB_1 or CB_2 receptors (Costa *et al*, 2004), although it has been reported that it does not elicit the decrease in blood pressure or increased respiration usually produced by TRPV1 activation by capsaicin or by the endocannabinoid anandamide (McQueen *et al*, 2004). Cannabidiol inhibits the hydrolysis of anandamide in mouse brain microsomes (Watanabe *et al*, 1996; Bisogno *et al*, 2001) and the carrier-mediated cellular uptake of anandamide in mast cells (Rakhshan *et al*, 2000; Bisogno *et al*, 2001). This suggests that administration of cannabidiol may enhance the activity of endogenous anandamide, although the (+)-stereoisomer and other cannabidiol analogues display more potent inhibition of anandamide inactivation than the natural isomer, (−)-cannabidiol, used in most pharmacological investigations (Bisogno *et al*, 2001).

Following the observation that cannabidiol reversed effects of Δ^9-THC in humans, such as anxiety (Karniol *et al*, 1974; Zuardi *et al*, 1982), research also examined its potential as an antipsychotic. Cannabidiol was reported to reverse some dopaminergic effects associated with apomorphine, such as stereotypy, prolactin secretion, and palpebral ptosis, while producing none of the catalepsy associated with 'typical' antipsychotics such as haloperidol (Zuardi *et al*, 1991). This group later showed that cannabidiol increased Fos protein expression in the nucleus accumbens, but not in the striatum, indicating that cannabidiol produces neuronal activation in mesolimbic areas but not in motor control areas, and thus reinforcing the potential of cannabidiol to produce few unwanted motor effects (Guimaraes *et al*, 2004). In humans, cannabidiol has been shown to reverse binocular depth inversion (a model of

*Correspondence: LE Long, Department of Pharmaceutical Biology and Pharmacology, Victorian College of Pharmacy, Monash University, 381 Royal Parade, Parkville, Victoria 3052, Australia, Tel: +61 3 9903 9085, Fax: +61 3 9903 9638, E-mail: leonora.long@vcp.monash.edu.au
Received 7 January 2005; revised 23 May 2005; accepted 7 June 2005
Online publication: 21 June 2005 at http://www.acnp.org/citations/Npp06210505001l/default.pdf

impaired visual perception during psychotic states) produced by the synthetic psychotropic cannabinoid nabilone (Leweke *et al*, 2000). Cannabidiol was also efficacious in reducing psychotic symptoms and well tolerated in a clinical trial in a young schizophrenic female patient (Zuardi *et al*, 1995). Most recently, cannabidiol was reported to reverse hyperlocomotion produced by amphetamine and ketamine in mice without producing catalepsy (Moreira and Guimaraes, 2005).

Prepulse inhibition (PPI) is an animal model of sensorimotor gating, and is defined as the decrease in the acoustic startle response when a nonstartling prepulse is presented 30–500 ms before the startling pulse (Hoffman and Ison, 1980). Sensorimotor gating is deficient in patients with psychotic disorders such as schizophrenia (Braff and Geyer, 1990). Pharmacological models of disrupted PPI include dopaminergic and serotonergic activation and NMDA receptor antagonism and are excellent predictors of antipsychotic activity (Geyer *et al*, 2001). Another form of startle response plasticity is habituation or the progressive reduction in response to an initially novel stimulus when the stimulus is presented repeatedly to a subject. The rate of habituation can be manipulated and is also reduced in psychotic disorders (Geyer *et al*, 1990; Bolino *et al*, 1992). Based on observations in the literature of the antipsychotic potential of cannabidiol, the aim of the following experiments was to test the ability of cannabidiol to reverse disruptions in PPI induced by the noncompetitive NMDA receptor antagonist MK-801 and to determine the involvement of TRPV1 receptors in the effects of cannabidiol using the TRPV1 antagonist capsazepine. Clozapine was administered as a positive control to test the effects of a known antipsychotic against those of cannabidiol in reversing MK-801-induced disruptions.

METHODS

Animals and Housing

Male Swiss mice weighing between 25 and 30 g were used. The animals were housed in group cages and kept at 22°C with a 12 h light–dark cycle. Food and drinking water were available *ad libitum*. The animal experimental protocols were approved by the Victorian College of Pharmacy, Monash University Animal Ethics Committee and conform to the guidelines set out by the National Health and Medical Research Council and all Australian Government regulations.

Drug Treatment

As a result of the number of interactions to be investigated, the injection schedule consisted of three injections. All animals were exposed to one treatment combination only.

Experiment 1: MK-801. Separate groups of mice were injected intraperitoneally (i.p.) with vehicle (1 : 1 : 98 Tween® 80 : EtOH : saline, VEH1) followed 20 min later by a second i.p. injection of vehicle (1 : 1 : 18 Cremophor® EL : EtOH : saline, VEH2). Then, 20 min after this, mice were given a third i.p. injection of MK-801 (0.1, 0.3, or 1 mg/kg, MK).

Experiment 2: Cannabidiol. Separate groups of mice were injected i.p. with vehicle (VEH1) followed 20 min later by an i.p. injection of cannabidiol (1, 5, or 15 mg/kg, CBD) and then followed 20 min later by a third i.p. injection of vehicle (0.1% ascorbic acid in distilled water, VEH3).

Experiment 3: Capsazepine + cannabidiol + MK-801. Separate groups of mice were injected i.p. with capsazepine (20 mg/kg, CPSZ) or vehicle (VEH1), followed 20 min later by i.p. cannabidiol (5 mg/kg, CBD) or vehicle (VEH2) and then followed by a third injection 20 min later of i.p. MK-801 (1 mg/kg) or vehicle (VEH3).

Experiment 4: Clozapine + MK-801. Separate groups of mice were injected i.p. with vehicle (VEH1) followed 20 min later by an i.p. injection of clozapine (4 mg/kg, CLOZ) and then followed 20 min later by a third i.p. injection of MK-801 (1 mg/kg) or vehicle (VEH3).

This regime of three injections ensured that all mice received the same number of injections. Thus the data from groups receiving VEH1 + VEH2 + VEH3, VEH1 + CBD 5 + VEH3, VEH1 + VEH2 + MK 1, and VEH1 + CBD 5 + MK 1 were reused in several analyses, as the appropriate vehicle control treatments were present.

Behavioural Testing

Startle reactivity was measured using two SR-LAB startle chambers (San Diego Instruments, San Diego, CA). The animal enclosures consisted of a perspex cylinder 40 mm in diameter on a platform connected to a piezoelectric accelerometer that detected movement within the cylinder. Above the cylinder was a speaker capable of producing white noise up to 120 dB(A) attached to programmable audio controls. The animal enclosure was located in an illuminated, ventilated, and sound-attenuated chamber.

All testing took place during the light phase. Animals were acclimatized in the startle chambers during three 0.5 h sessions: a morning and afternoon session on the day before testing and a morning session on the test day. In the afternoon of the test day, mice received three injections as described above. At 5 min after the third injection, mice were placed in the startle chamber. Mice were returned to their home cage between injections and before placement in the startle chamber.

After 5 min acclimatization to the background noise in the startle chamber of 70 dB(A), startle stimulus trials of 120 dB(A) intensity and 40 ms duration were applied, either alone or preceded by 100 ms with a prepulse of an intensity of 3, 6, or 12 dB(A) above background and 20 ms duration. Prepulse alone trials of 3, 6, or 12 dB(A) above background were also presented, as were trials containing no stimulus at all. A total of 10 trials of each type were presented in a pseudorandom order, with the intertrial interval varying in a random fashion from 8 to 22 s. An extra 10 pulse alone trials were presented in blocks at the beginning and end of each test session in order to observe habituation of the startle response and to scale down the initial startle response to a stable plateau. The whole body flinch (movement) elicited by the startle stimulus was detected by the accelerometer. PPI was calculated as a percentage of

this startle response using the formula: % PPI = (1−(startle amplitude after prepulse-pulse pair/startle amplitude after pulse only)) × 100. A 0% PPI value indicates that there is no difference between the startle response (movement) to prepulse-plus-pulse trials and pulse alone trials. Positive values indicate the extent to which the startle response is diminished in the presence of a prepulse. Habituation was calculated according to the formula: % habituation = (1−(startle amplitude at end of test session/startle amplitude at beginning of test session)) × 100. A positive % habituation value indicates that a decrease in startle response has occurred over time.

Drugs

The following drugs were used: (−)-cannabidiol (Tocris), capsazepine (Sigma, Australia), clozapine (obtained from Dr Ben Capuano, Monash University), MK-801 (Sigma, Australia), Tween® 80 (Sigma, Australia), Cremophor® EL (BASF), and ascorbic acid (David Craig Galenicals, Australia).

The injection volume in each mouse for each drug was 10 ml/kg.

Statistical Analysis

A one-way ANOVA was used to compare both startle responses and % habituation between treatment groups in Experiments 1 and 2. When a main effect of treatment was detected ($P<0.05$), a Dunnett's *vs* control *post hoc* test was used to determine the level of significance for each treatment group. Differences between both startle responses and % habituation in Experiment 3 were measured using $2 \times 2 \times 2$ ANOVA between treatment groups. Four *post hoc* individual planned contrasts between drug treatment combinations of interest were carried out using $\alpha = 0.0125$ ($\alpha = 0.05/4$).

A one-way repeated measures ANOVA was used to compare % PPI between groups in Experiments 1 and 2 (MK-801 and cannabidiol dose–response curves) with four levels of the between-subjects factor (0, 0.1, 0.3, or 1 mg/kg MK-801 or 0, 1, 5, or 15 mg/kg cannabidiol for Experiments 1 and 2, respectively) and prepulse intensity (3, 6, or 12 dB(A) above background) as the within-subjects factor. When a main effect of treatment on PPI was detected ($P<0.05$), a Dunnett's *vs* control *post hoc* test was performed to evaluate significant differences.

A $2 \times 2 \times 2$ factorial ANOVA was used to compare % PPI between groups in Experiment 3. There were three between-subjects factors (capsazepine or vehicle, cannabidiol or vehicle, and MK-801 or vehicle) and prepulse intensity (3, 6, or 12 dB(A) above background) was the within-subjects factor. When a main effect of treatment on PPI was detected ($P<0.05$), four *post hoc* individual planned contrasts between drug treatment combinations of interest were carried out using $\alpha = 0.0125$.

A 2×2 repeated measures ANOVA was used to compare % PPI between groups in Experiment 4. There were two between-subjects factors (clozapine or vehicle and MK-801 or vehicle) and prepulse intensity (3, 6, or 12 dB(A) above background) was the within-subjects factor. Two *post hoc* individual planned contrasts were carried out using $\alpha = 0.025$ ($\alpha = 0.05/2$).

In all % PPI data analyses, when a drug treatment × prepulse intensity interaction was detected, *post hoc* ANOVA was performed at each level of prepulse intensity.

Statistical analyses were performed with SPSS 11.5 for Windows (SPSS Inc.; Chicago, USA).

RESULTS

Experiment 1: MK-801

Statistical analysis revealed a significant effect on the startle response of VEH1 + VEH2 + MK 0.1 and VEH1 + VEH2 + MK 0.3, but not VEH1 + VEH2 + MK 1 treatment, in comparison with vehicle ($F_{3,23} = 9.595$, $P<0.001$, $n = 6$–8, Figure 1a).

There was a significant main effect of MK-801 on PPI ($F_{3,23} = 8.044$, $P<0.01$, $n = 6$–8, Figure 1b). There was a significant effect of prepulse intensity, reflecting increased PPI in the presence of greater prepulse intensities ($F_{2,46} = 12.105$, $P<0.001$, $n = 6$–8). There was a significant treatment × prepulse intensity interaction ($F_{6,46} = 3.400$, $P<0.01$, $n = 6$–8). *Post hoc* ANOVA was performed to analyse % PPI data at each prepulse intensity, with Dunnett's test showing a significant effect at 12 dB for the VEH1 + VEH2 + MK 0.3 treatment group ($F_{3,23} = 4.201$, $P<0.05$, $n = 6$–8) and at 6 and 12 dB for the VEH1 + VEH2 + MK 1 treatment group ($F_{3,23} = 10.585$, $P<0.001$, $n = 6$–8).

There was no significant effect of MK-801 on habituation ($P>0.05$, ANOVA, Table 1).

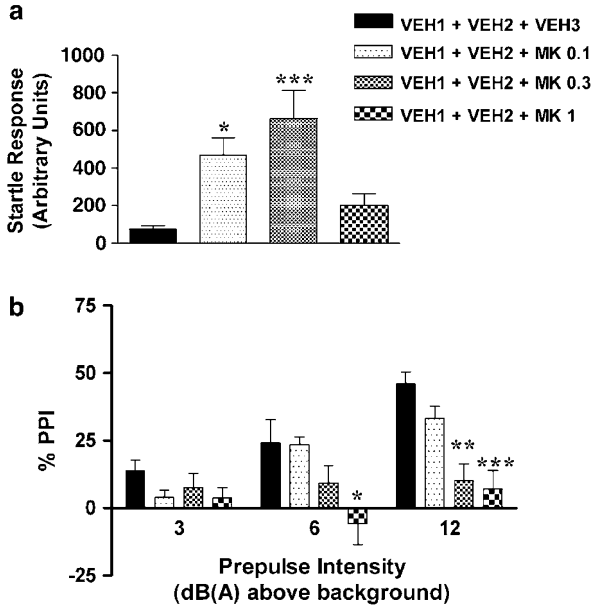

Figure 1 Effect of MK-801 (0.1, 0.3, or 1 mg/kg) following pretreatment with vehicles (VEH1, 40 min beforehand; VEH2, 20 min beforehand) on (a) acoustic startle response and (b) prepulse inhibition (PPI) of the startle response in mice. Results are expressed as mean ± SEM. $n = 6$–8. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ *vs* vehicle treatment group (Dunnett's test). MK = MK-801, VEH1 = 1 : 1 : 98 Tween® 80 : EtOH : saline, VEH2 = 1 : 1 : 18 Cremophor® EL : EtOH: saline, VEH3 = 0.1% ascorbic acid in distilled water.

Table 1 Effect of Capsazepine (20 mg/kg) or Vehicle (VEH1) Administered 20 min Prior to Cannabidiol (1, 5, or 15 mg/kg) or Clozapine (4 mg/kg) or Vehicle (VEH2), and 40 min Prior to MK-801 (0.1, 0.3, or 1 mg/kg) or Vehicle (VEH3)

Treatment	% Habituation	Treatment	% Habituation
VEH1+VEH2+VEH3	−8.9+18.0	VEH1+CBD 5+MK 1	0.3±15.1
VEH1+VEH2+MK 0.1	5.6±13.2	CPSZ 20+CBD 5+MK 1	10.6±13.5
VEH1+VEH2+MK 0.3	−19.6±16.8	CPSZ 20+VEH2+MK 1	−27.5±18.9
VEH1+VEH2+MK 1	31.5±12.6	CPSZ 20+CBD 5+VEH3	36.6±15.2
VEH1+CBD 1+VEH3	22.2±9.5	CPSZ 20+VEH2+VEH3	14.8±7.6
VEH1+CBD 5+VEH3	20.1±5.6	VEH1+CLOZ 4+VEH3	13.5±9.8
VEH1+CBD 15+VEH3	18.0±4.6	VEH1+CLOZ 4+MK 1	33.8±9.9

Data are presented as the mean (±SEM) % habituation in mice. VEH1 = 1 : 1 : 98 Tween® 80 : EtOH : saline, VEH2 = 1 : 1 : 18 Cremophor® EL : EtOH : saline, VEH3 = 0.1% ascorbic acid in distilled water.

These results indicate that MK-801 significantly increased the startle response at lower doses (0.1 and 0.3 mg/kg). MK-801 disrupted PPI at higher doses (0.3 and 1 mg/kg) but did not affect habituation.

Experiment 2: Cannabidiol

There was a significant effect on startle response of VEH1 + CBD 1 + VEH3 and VEH1 + CBD 15 + VEH3, but not VEH1 + CBD 5 + VEH3 treatment, in comparison with vehicle ($F_{3,21} = 9.383$, $P < 0.001$, $n = 6$–7, Figure 2a).

There was no significant effect of cannabidiol (1, 5, or 15 mg/kg) on PPI ($P > 0.05$, Figure 2b). There was a significant effect of prepulse intensity, reflecting increased PPI in the presence of greater prepulse intensities ($F_{1.55,32.51} = 78.887$, $P < 0.001$, $n = 6$–7).

There was no significant effect of cannabidiol on habituation ($P > 0.05$, ANOVA, Table 1).

These results indicate that cannabidiol significantly increased the startle response at the lowest and highest dose administered (1 and 15 mg/kg) but had no effect on PPI or habituation when administered with vehicle alone.

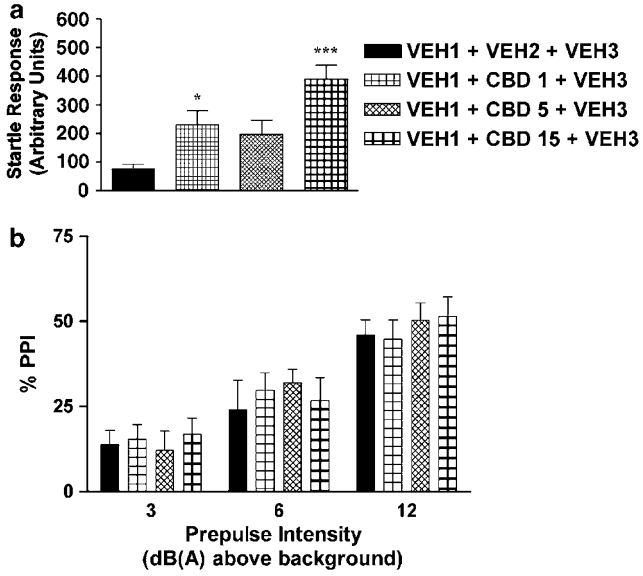

Figure 2 Effect of cannabidiol (1, 5, or 15 mg/kg) following pretreatment with vehicle (VEH1, 20 min beforehand) and 20 min prior to a second vehicle treatment (VEH3) on (a) acoustic startle response and (b) prepulse inhibition (PPI) of the startle response in mice. Results are expressed as mean ± SEM. $n = 6$–7. *$P < 0.05$, ***$P < 0.001$ *vs* vehicle treatment group (Dunnett's test). CBD = cannabidiol, VEH1 = 1 : 1 : 98 Tween® 80 : EtOH : saline, VEH2 = 1 : 1 : 18 Cremophor® EL : EtOH: saline, VEH3 = 0.1% ascorbic acid in distilled water.

Experiment 3: Capsazepine + Cannabidiol + MK-801

There was a significant main effect of capsazepine on startle response ($F_{1,42} = 4.542$, $P < 0.05$, $n = 5$–8) and a main effect of MK-801 on startle response ($F_{1,42} = 5.698$, $P < 0.05$, $n = 5$–8, Figure 3a). There was a significant interaction between the effects of capsazepine and cannabidiol on startle response ($F_{1,42} = 6.091$, $P < 0.05$, $n = 5$–8). Individual planned comparisons did not reveal a significant effect of MK-801 on startle response compared with vehicle ($P > 0.05$, VEH1 + VEH2 + VEH3 *vs* VEH1 + VEH2 + MK 1) nor any effect of cannabidiol or capsazepine on the startle response in MK-801-treated mice ($P > 0.05$, CPSZ 20 + VEH2 + MK *vs* VEH1 + VEH2 + MK 1 and VEH1 + CBD 5 + MK 1 *vs* VEH1 + VEH2 + MK 1).

There was a main effect of capsazepine ($F_{1,42} = 4.919$, $P < 0.05$, $n = 5$–8) and of MK-801 ($F_{1,42} = 8.832$, $P < 0.01$, $n = 5$–8, Figure 3b) on PPI. There was a significant capsazepine × cannabidiol interaction ($F_{1,42} = 4.178$, $P < 0.05$, $n = 5$–8). There was a significant effect of prepulse intensity, reflecting increased PPI in the presence of greater prepulse intensities ($F_{2,84} = 38.071$, $P < 0.001$, $n = 5$–8). There was also a significant prepulse intensity × MK-801 interaction ($F_{2,84} = 7.723$, $P < 0.01$, $n = 5$–8) and a significant prepulse intensity × capsazepine × cannabidiol × MK-801 interaction ($F_{2,84} = 5.393$, $P < 0.01$, $n = 5$–8).

Owing to the interactions between prepulse intensity and drug treatment, % PPI data were analysed by three-way ANOVA at each level of prepulse intensity. There was no significant effect of drug treatment at 3 dB. At 6 dB, there was a significant effect of MK-801 ($F_{1,42} = 16.088$, $P < 0.001$, $n = 5$–8) and a significant capsazepine × cannabidiol interaction ($F_{1,42} = 4.715$, $P < 0.05$, $n = 5$–8). At 12 dB, there were significant effects of capsazepine ($F_{1,42} = 6.147$, $P < 0.05$, $n = 5$–8) and MK-801 ($F_{1,42} = 8.507$, $P < 0.01$, $n = 5$–8) and a significant capsazepine × cannabidiol interaction ($F_{1,42} = 5.150$, $P < 0.05$, $n = 5$–8). At both 6 dB and 12 dB, the

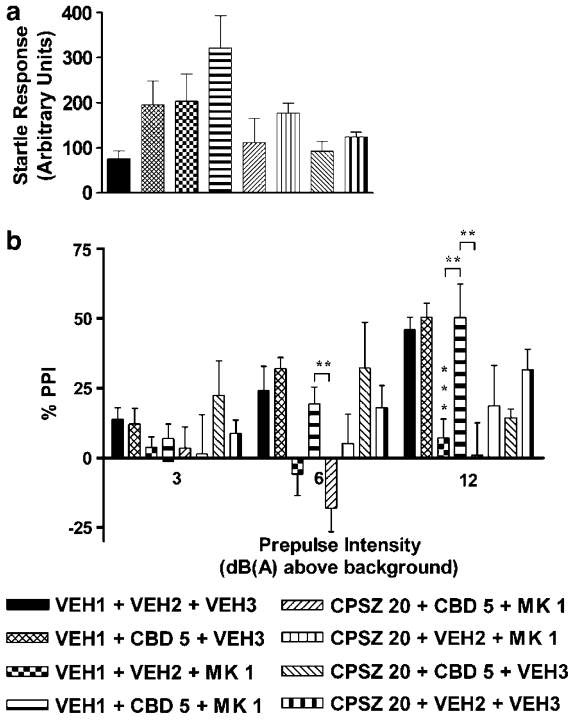

Figure 3 Effect of pretreatment with capsazepine (20 mg/kg) 20 min prior to cannabidiol (5 mg/kg) and 40 min prior to MK-801 (1 mg/kg) on (a) acoustic startle response and (b) prepulse inhibition (PPI) of the startle response in mice. Results are expressed as mean ± SEM. $n = 5–8$. $^{**}P < 0.01$ between treatment groups as indicated, $^{***}P < 0.001$ *vs* vehicle treatment group (individual planned comparisons, $\alpha = 0.0125$). CPSZ = capsazepine, CBD = cannabidiol, MK = MK-801, VEH1 = 1 : 1 : 98 Tween® 80 : EtOH: saline, VEH2 = 1 : 1 : 18 Cremophor® EL : EtOH: saline, VEH3 = 0.1% ascorbic acid in distilled water.

capsazepine × cannabidiol × MK-801 interaction approached significance ($P = 0.067$ and $P = 0.052$ respectively).

Individual planned comparisons between treatment groups at each level of prepulse intensity revealed significant differences in PPI between VEH1 + VEH2 + VEH3 and VEH1 + VEH2 + MK 1 at the 12 dB prepulse level ($t_{0.0125,28} = 4.689$, $P < 0.001$), between VEH1 + VEH2 + MK 1 and VEH1 + CBD 5 + MK 1 at the 12 dB level ($t_{0.0125,28} = 3.111$, $P < 0.01$), and between VEH1 + CBD 5 + MK 1 and CPSZ 20 + CBD 5 + MK 1 at the 6 dB ($t_{0.0125,28} = 3.623$, $P < 0.01$) and 12 dB levels ($t_{0.0125,28} = 2.946$, $P < 0.01$). There was no significant difference between CPSZ 20 + VEH2 + MK 1 and VEH1 + VEH2 + MK 1 at any of the three prepulse intensities ($P > 0.05$).

There were no significant main effects of capsazepine, cannabidiol, or MK-801 on % habituation ($P > 0.05$, Table 1). There was a significant capsazepine × MK-801 interaction ($F_{1,42} = 4.835$, $P < 0.05$, $n = 5–8$, Table 1).

These results indicate that capsazepine, cannabidiol, and MK-801 administered with vehicle or in combination did not alter the startle response. The disruption in PPI induced by MK-801 (1 mg/kg) was significantly restored by cannabidiol (5 mg/kg) and this effect was reversed by pretreatment with capsazepine (20 mg/kg). Capsazepine alone did not reverse the MK-801-induced PPI disruption.

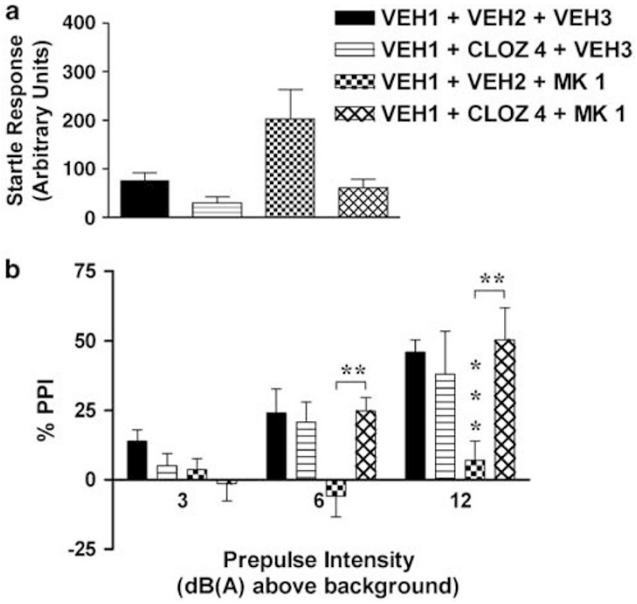

Figure 4 Effect of clozapine (4 mg/kg) following pretreatment with vehicle (VEH1, 20 min beforehand) and 20 min prior to MK-801 (1 mg/kg) on (a) acoustic startle response and (b) prepulse inhibition (PPI) of the startle response in mice. Results are expressed as mean ± SEM $n = 5–8$. $^{**}P < 0.01$ between treatment groups as indicated, $^{***}P < 0.001$ *vs* vehicle treatment group (individual planned comparisons, $\alpha = 0.0125$). CLOZ = clozapine, MK = MK-801, VEH1 = 1 : 1 : 98 Tween® 80 : EtOH : saline, VEH2 = 1 : 1 : 18 Cremophor® EL : EtOH : saline, VEH3 = 0.1% ascorbic acid in distilled water.

Experiment 4: Clozapine + MK-801

There was a main effect of clozapine on startle response ($F_{1,22} = 5.306$, $P < 0.05$, $n = 5–8$, Figure 4a). The effect of MK-801 on startle response approached significance ($P = 0.069$) but there was no significant clozapine × MK-801 interaction ($P > 0.05$). However, *post hoc* analysis revealed no significant difference in startle response between the VEH1 + VEH2 + VEH3 and either the VEH1 + CLOZ 4 + VEH3 or the VEH1 + CLOZ 4 + MK 1 treatment groups.

There was a main effect of MK-801, but not clozapine, on PPI ($F_{1,22} = 5.034$, $P < 0.05$, $n = 5–8$, Figure 4b). There was a significant clozapine × MK-801 interaction ($F_{1,22} = 8.305$, $P < 0.01$, $n = 5–8$). There was a significant effect of prepulse intensity, reflecting increased PPI in the presence of greater prepulse intensities ($F_{2,44} = 20.566$, $P < 0.001$, $n = 5–8$). There was also a significant prepulse intensity × clozapine interaction ($F_{2,44} = 3.883$, $P < 0.05$, $n = 5–8$).

Post hoc three-way ANOVA was performed to analyse % PPI data at each level of prepulse intensity. At 6 and 12 dB, there were significant clozapine × MK-801 interactions ($F_{1,22} = 4.803$, $P < 0.05$ and $F_{1,22} = 7.471$, $P < 0.05$, respectively, $n = 5–8$). Individual planned comparisons at each level of prepulse intensity revealed significant differences in PPI between VEH1 + VEH2 + VEH3 and VEH1 + VEH2 + MK 1 at the 12 dB prepulse level ($t_{0.025,19} = 4.689$, $P < 0.001$) and between VEH1 + VEH2 + MK 1 and VEH1 + CLOZ 4 + MK 1 at the 6 dB ($t_{0.025,19} = 3.335$, $P < 0.01$) and 12 dB levels ($t_{0.025,19} = 3.275$, $P < 0.01$).

There was a main effect of MK-801, but not clozapine, on habituation ($F_{1,22} = 6.156$, $P < 0.05$, $n = 5$–8, Table 1) but there was no significant clozapine × MK-801 interaction ($P > 0.05$). However, *post hoc* analysis revealed no significant difference in habituation between the VEH1 + VEH2 + VEH3 and either the VEH1 + VEH2 + MK 1 or the VEH1 + CLOZ 4 + MK 1 treatment groups.

These results show that clozapine (4 mg/kg) decreased the startle response when administered with vehicle. This dose of clozapine did not disrupt PPI when administered with vehicle but did reverse the disruption in PPI induced by MK-801 (1 mg/kg).

DISCUSSION

The present results demonstrate that cannabidiol reverses MK-801-induced disruption of PPI. MK-801 dose-dependently disrupted PPI with a concomitant increase in the magnitude of the startle response at the lower and intermediate doses. Cannabidiol did not affect PPI when administered with vehicle alone, although it did increase the startle response. Cannabidiol reversed the disruption in PPI elicited by the highest dose of MK-801, an effect blocked by pretreatment with capsazepine prior to cannabidiol and MK-801. Clozapine also reversed the MK-801-induced disruption of PPI, while capsazepine alone did not. To our knowledge, this is the first report that cannabidiol can restore sensorimotor gating deficits induced by MK-801.

The disruption of PPI elicited by MK-801 is consistent with previous reports of MK-801-induced disruption of PPI in mice (Curzon and Decker, 1998; Sakaue *et al*, 2003; Yee *et al*, 2004). It has been proposed that blockade of NMDA receptor-mediated transmission by noncompetitive antagonists such as MK-801 leads to excessive stimulation of non-NMDA glutamate-gated ion channels such as α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainic acid (KA) receptors via reduced stimulation of GABAergic interneurons and subsequent disinhibition of glutamatergic neurons (Deutsch *et al*, 2001). This effect may be manifest in schizophrenia as NMDA receptor hypofunction (NRH), leading not only to excitotoxic activation of AMPA/KA receptors but also to dysregulation of dopaminergic balance over the long term by diminishing cortical dopaminergic tone and thus increasing subcortical dopaminergic tone (Deutsch *et al*, 2001). However, modulation of dopaminergic activity is unlikely to be directly responsible for the disruptive effect of MK-801 on PPI, as MK-801 also disrupted PPI in wild-type and mutant D_1 and D_2 receptor knockout mice (Ralph-Williams *et al*, 2002), and typical antipsychotics with dopamine antagonist profiles do not restore MK-801-induced deficits in sensorimotor gating (Curzon and Decker, 1998; Martin *et al*, 2003). In the present study, the observation that the atypical antipsychotic clozapine restores MK-801-induced PPI disruption is in agreement with previous studies in rats and mice (Bakshi *et al*, 1994; Martin *et al*, 2003; Bubenikova *et al*, 2005) and provides a positive control for reversal of MK-801-induced sensorimotor gating deficits.

The startle response tended to be increased in some treatment groups such as the VEH1 + CBD 5 + MK 1 group. The effects of centrally active drugs on startle response and PPI in mice tend to be dissociated (Geyer *et al*, 2002), reflecting the relative simplicity of the neurocircuitry of the startle response and the more complex neural mechanisms regulating PPI. This dissociation is reflected in our data, in which a slightly increased startle response and disrupted PPI was observed in the VEH1 + VEH2 + MK 1 group while a similarly increased startle response and normal PPI was observed in the VEH1 + CBD 5 + VEH3 group.

The restorative effect of cannabidiol on MK-801-induced disruption reflects an antipsychotic potential of cannabidiol. This is in keeping with similar indications observed in rats in which cannabidiol reversed apomorphine-induced stereotypy (Zuardi *et al*, 1991) and in a female schizophrenic patient in whom cannabidiol markedly reduced psychotic symptoms (Zuardi *et al*, 1995). The dose range of cannabidiol (1, 5, and 15 mg/kg) used in the present study is lower than doses previously determined to reverse the effects of psychoactive drugs in rats (Zuardi *et al*, 1991; Moreira and Guimaraes, 2005); however, it has previously been shown to be active in extinguishing conditioned place preference learning induced by amphetamine and cocaine (Parker *et al*, 2004). The lack of effect of either capsazepine or cannabidiol *per se* on PPI and startle response suggests that these drugs do not directly interfere with normal sensorimotor gating in the mouse. Importantly, the lack of effect of cannabidiol on startle reflects the comparative absence of sedative or cataleptic effects at these doses of cannabidiol.

There was no difference between the habituation observed in any of the treatment groups, suggesting that this form of startle response plasticity is not affected by TRPV1 or NMDA receptor antagonism or by cannabidiol in the present study. MK-801 has previously been reported to impair habituation in mice (Klamer *et al*, 2004). It has been suggested that separating habituation test sessions comprising consecutive startling pulses by a PPI session consisting of both startling pulses and prepulses may influence the degree of habituation (Varty *et al*, 2000). The present study used this experimental protocol which may explain the difference between the present results and the results of the Klamer *et al* study, which used a series of 121 consecutive pulse-alone trials.

In vitro studies have reported that cannabidiol has affinity for TRPV1 receptors and inhibits the hydrolysis and cellular uptake of the endogenous cannabinoid anandamide (Bisogno *et al*, 2001). Pretreatment with the TRPV1 receptor antagonist capsazepine prior to cannabidiol and MK-801 elicited a disruption of PPI similar to that observed with vehicle and MK-801 alone, suggesting that TRPV1 receptors may be involved in the restorative effect of cannabidiol on MK-801-induced disruption of PPI. However, although capsazepine has been shown to reverse capsaicin-induced effects such as dopaminergic cell death induced *in vivo* by intranigral injection (Kim *et al*, 2005) and to reverse antihyperalgesic effects of cannabidiol in rats (Costa *et al*, 2004), it has also been shown to block calcium channels and nicotinic cholinergic receptors at the same doses at which it blocks the TRPV1 receptor (Docherty *et al*, 1997; Liu and Simon, 1997), making it difficult to conclusively identify the involvement of TRPV1 receptors in the effect of cannabidiol in the present study. In preliminary experiments in our laboratory with systemic administration of the potent and

selective TRPV1 antagonist iodoresiniferatoxin, hypomotility and accompanying hypothermia (unpublished observations) resulted in the startle response being too low for use in obtaining meaningful PPI data.

Following the detection of the expression of TRPV1 receptors in areas including the cortex, hippocampus, central amygdala, striatum, hypothalamus, substantia nigra, reticular formation and cerebellum (Mezey *et al*, 2000) and the localization of TRPV1 receptors on neurons, astrocytes, and pericytes (Toth *et al*, 2005), interest in the role of TRPV1 receptors has broadened from a focus on their role in pain perception into consideration of their involvement in the control of emotions, learning, and satiety. These brain regions are also part of the circuitry regulating sensorimotor gating (Koch and Schnitzler, 1997; Swerdlow and Geyer, 1998). From the observation that TRPV1 and tyrosine hydroxylase expression are colocalized in the substantia nigra, it was suggested that vanilloid-sensitive neurons are monoaminergic in this region (Mezey *et al*, 2000). Expression of TRPV1 receptors on glutamatergic terminals is also likely as their activation produces glutamate release in the rat hypothalamus (Sasamura *et al*, 1998), substantia nigra (Marinelli *et al*, 2003), and substantia gelatinosa (Yue *et al*, 2004). Further work has demonstrated that activation of TRPV1 receptors in the ventral tegmental area causes dopamine release in the nucleus accumbens, which may be due to increased glutamatergic transmission onto dopaminergic neurons (Marinelli *et al*, 2005). The existence of TRPV1 receptors in brain regions involved in sensorimotor gating is in agreement with the results obtained in the present study.

Anandamide has been proposed as an endogenous TRPV1 receptor agonist (Szallasi and Di Marzo, 2000). It activates TRPV1 receptors and facilitates neurotransmitter release (Zygmunt *et al*, 1999; Al-Hayani *et al*, 2001; Marinelli *et al*, 2003; for a review, see Ross, 2003). The efficacy of anandamide as a TRPV1 agonist varies with receptor reserve, cellular uptake, fatty acid amide hydrolase (FAAH) and lipoxygenase metabolism, and protein signalling pathways (Ross, 2003; Van Der Stelt and Di Marzo, 2004) and may be increased in pathological conditions such as inflammation in which upregulation of TRPV1 receptors occurs. An entourage effect of *N*-acyl ethanolamides such as palmitoylethylamide coreleased with anandamide may facilitate the activity of anandamide at TRPV1 receptors (De Petrocellis *et al*, 2001; Smart *et al*, 2002). In the CNS, anandamide is present in brain regions expressing TRPV1 receptors including hippocampus and basal ganglia, regions also expressing high levels of CB_1 receptor, for which anandamide is also an endogenous agonist.

Retrograde signalling of endocannabinoids involves postsynaptic synthesis and release following depolarization of the postsynaptic terminal and diffusion across the synaptic cleft to bind to presynaptic CB_1 receptors (Wilson and Nicoll, 2001). As the effect of anandamide on both TRPV1 and CB_1 receptors is regulated by FAAH hydrolysis and cellular uptake by the putative anandamide transporter (Hillard, 2000), it is possible that inhibition of anandamide hydrolysis and reuptake by cannabidiol could lead to potentiation of the activity of anandamide at both TRPV1 and CB_1 receptors. Coexpression of CB_1 and TRPV1 receptors has been demonstrated in rat dorsal root ganglion neurons (Ahluwalia *et al*, 2000; Bridges *et al*, 2003) and in rat mesencephalic cultures (Kim *et al*, 2005). Tonic activation of CB_1 receptors exerts an inhibitory control over TRPV1 receptors, as anandamide or capsaicin-induced TRPV1 receptor-mediated effects are potentiated in the presence of a CB_1 receptor antagonist (Maccarrone *et al*, 2000; Mang *et al*, 2001; Lever and Malcangio, 2002). Furthermore, cell death induced by capsaicin and the CB_1 agonist HU-210 in rat mesencephalic culture was reversed by the CB_1 receptor antagonist AM 251 and capsazepine, respectively, suggesting functional crosstalk between the two receptors (Kim *et al*, 2005). Cannabidiol produces TRPV1 activation at a nine-fold lower concentration than that at which it inhibits anandamide hydrolysis and at a seven-fold lower concentration than that at which it blocks cellular anandamide uptake (Bisogno *et al*, 2001), thus CB_1 receptor-mediated effects resulting from inhibition of anandamide hydrolysis are likely to be counteracted by the direct activation of TRPV1 by cannabidiol. If cannabidiol exerted its effect on disrupted PPI in the present study via TRPV1 receptor activation, changes in mesolimbic glutamatergic and dopaminergic transmission may have occurred, thus counteracting the effect of NMDA receptor antagonism by MK-801.

The present results are of further interest in the context of the epidemiological relationship observed between cannabis consumption and schizophrenia. Individuals who consume cannabis are twice as likely to develop schizophrenia (Smit *et al*, 2004), although evidence does not suggest that cannabis consumption independently precipitates schizophrenia, but that it may be a companion to a complex group of factors predisposing an individual to the development of the disease (Arseneault *et al*, 2004). Thus although Δ^9-THC and other psychotropic cannabinoids may be precipitating risk factors for schizophrenia, the present results provide scope for speculation on the potential role of the nonpsychotropic *Cannabis* constituent cannabidiol as an antipsychotic compound.

In summary, the present study is the first to investigate the effects of cannabidiol and capsazepine on PPI and suggests a promising avenue for investigation into the central effects of cannabidiol. The results indicate that cannabidiol reverses sensorimotor gating deficits induced by the NMDA receptor antagonist MK-801, and that this effect may be mediated by TRPV1 receptors as evidenced by the reversal of the effect of cannabidiol by capsazepine. Future studies using more selective ligands and several models of disrupted PPI will further elucidate the mechanism of the effects of cannabidiol on PPI.

ACKNOWLEDGEMENTS

We thank Dr Aidan Sudbury and Dr Bill Thiel (Monash University) for their invaluable assistance in statistical analysis of PPI data and Dr Ben Capuano (Monash University) for his synthesis and provision of clozapine.

REFERENCES

Ahluwalia J, Urban L, Capogna M, Bevan S, Nagy I (2000). Cannabinoid 1 receptors are expressed in nociceptive primary sensory neurons. *Neuroscience* **100**: 685–688.

Al-Hayani A, Wease KN, Ross RA, Pertwee RG, Davies SN (2001). The endogenous cannabinoid anandamide activates vanilloid receptors in the rat hippocampal slice. *Neuropharmacology* **41**: 1000–1005.

Arseneault L, Cannon M, Witton J, Murray RM (2004). Causal association between cannabis and psychosis: examination of the evidence. *Br J Psychiatry* **184**: 110–117.

Bakshi VP, Swerdlow NR, Geyer MA (1994). Clozapine antagonizes phencyclidine-induced deficits in sensorimotor gating of the startle response. *J Pharmacol Exp Ther* **271**: 787–794.

Bisogno T, Hanus L, De Petrocellis L, Tchilibon S, Ponde DE, Brandi I *et al* (2001). Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *Br J Pharmacol* **134**: 845–852.

Bolino F, Manna V, Di Cicco L, Di Michele V, Daneluzzo E, Rossi A *et al* (1992). Startle reflex habituation in functional psychoses: a controlled study. *Neurosci Lett* **145**: 126–128.

Braff DL, Geyer MA (1990). Sensorimotor gating and schizophrenia. Human and animal model studies. *Arch Gen Psychiatry* **47**: 181–188.

Bridges D, Rice ASC, Egertova M, Elphick MR, Winter J, Michael GJ (2003). Localisation of cannabinoid receptor 1 in rat dorsal root ganglion using *in situ* hybridisation and immunohistochemistry. *Neuroscience* **119**: 803–812.

Bubenikova V, Votava M, Horacek J, Palenicek T, Dockery C (2005). The effect of zotepine, risperidone, clozapine and olanzapine on MK-801-disrupted sensorimotor gating. *Pharmacol Biochem Behavior* **80**: 591–596.

Costa B, Giagnoni G, Franke C, Trovato AE, Colleoni M (2004). Vanilloid TRPV1 receptor mediates the antihyperalgesic effect of the nonpsychoactive cannabinoid, cannabidiol, in a rat model of acute inflammation. *Br J Pharmacol* **143**: 247–250.

Curzon P, Decker MW (1998). Effects of phencyclidine (PCP) and (+)MK-801 on sensorimotor gating in CD-1 mice. *Prog Neuropsychopharmacol Biol Psychiatry* **22**: 129–146.

De Petrocellis L, Davis JB, Di Marzo V (2001). Palmitoylethanolamide enhances anandamide stimulation of human vanilloid VR1 receptors. *FEBS Lett* **506**: 253–256.

Deutsch SI, Rosse RB, Schwartz BL, Mastropaolo J (2001). A revised excitotoxic hypothesis of schizophrenia: therapeutic implications. *Clin Neuropharmacol* **24**: 43–49.

Docherty RJ, Yeats JC, Piper AS (1997). Capsazepine block of voltage-activated calcium channels in adult rat dorsal root ganglion neurones in culture. *Br J Pharmacol* **121**: 1461–1467.

French ED, Dillon K, Wu X (1997). Cannabinoids excite dopamine neurons in the ventral tegmentum and substantia nigra. *Neuro Report* **8**: 649–652.

Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR (2001). Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology* **156**: 117–154.

Geyer MA, McIlwain KL, Paylor R (2002). Mouse genetic models for prepulse inhibition: an early review. *Mol Psychiatry* **7**: 1039–1053.

Geyer MA, Swerdlow NR, Mansbach RS, Braff DL (1990). Startle response models of sensorimotor gating and habituation deficits in schizophrenia. *Brain Res Bull* **25**: 485–498.

Guimaraes VM, Zuardi AW, Del Bel EA, Guimaraes FS (2004). Cannabidiol increases Fos expression in the nucleus accumbens but not in the dorsal striatum. *Life Sci* **75**: 633–638.

Hershkowitz M, Szechtman H (1979). Pre-treatment with delta 1-tetrahydrocannabinol and psychoactive drugs: effects on uptake of biogenic amines and on behavior. *Eur J Pharmacol* **59**: 267–276.

Hillard CJ (2000). Biochemistry and pharmacology of the endocannabinoids arachidonylethanolamide and 2-arachidonylglycerol. *Prostaglandins Other Lipid Mediat* **61**: 3–18.

Hoffman HS, Ison JR (1980). Reflex modification in the domain of startle: I. Some empirical findings and their implications for how the nervous system processes sensory input. *Psychol Rev* **87**: 175–189.

Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA *et al* (2002). International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev* **54**: 161–202.

Karniol IG, Shirakawa I, Kasinski N, Pfeferman A, Carlini EA (1974). Cannabidiol interferes with the effects of delta 9—tetrahydrocannabinol in man. *Eur J Pharmacol* **28**: 172–177.

Kim SR, Lee da Y, Chung ES, Oh UT, Kim SU, Jin BK (2005). Transient receptor potential vanilloid subtype 1 mediates cell death of mesencephalic dopaminergic neurons *in vivo* and *in vitro*. *J Neurosci* **25**: 662–671.

Klamer D, Palsson E, Revesz A, Engel JA, Svensson L (2004). Habituation of acoustic startle is disrupted by psychotomimetic drugs: differential dependence on dopaminergic and nitric oxide modulatory mechanisms. *Psychopharmacology* **176**: 440–450.

Koch M, Schnitzler HU (1997). The acoustic startle response in rats—circuits mediating evocation, inhibition and potentiation. *Behav Brain Res* **89**: 35–49.

Lever IJ, Malcangio M (2002). CB(1) receptor antagonist SR141716A increases capsaicin-evoked release of Substance P from the adult mouse spinal cord. *Br J Pharmacol* **135**: 21–24.

Leweke FM, Schneider U, Radwan M, Schmidt E, Emrich HM (2000). Different effects of nabilone and cannabidiol on binocular depth inversion in man. *Pharmacol Biochem Behavior* **66**: 175–181.

Liu L, Simon SA (1997). Capsazepine, a vanilloid receptor antagonist, inhibits nicotinic acetylcholine receptors in rat trigeminal ganglia. *Neurosci Lett* **228**: 29–32.

Maccarrone M, Lorenzon T, Bari M, Melino G, Finazzi-Agro A (2000). Anandamide induces apoptosis in human cells via vanilloid receptors. Evidence for a protective role of cannabinoid receptors. *J Biol Chem* **275**: 31938–31945.

Mang CF, Erbelding D, Kilbinger H (2001). Differential effects of anandamide on acetylcholine release in the guinea-pig ileum mediated via vanilloid and non-CB1 cannabinoid receptors. *Br J Pharmacol* **134**: 161–167.

Marinelli S, Di Marzo V, Berretta N, Matias I, Maccarrone M, Bernardi G *et al* (2003). Presynaptic facilitation of glutamatergic synapses to dopaminergic neurons of the rat substantia nigra by endogenous stimulation of vanilloid receptors. *J Neurosci* **23**: 3136–3144.

Marinelli S, Pascucci T, Bernardi G, Puglisi-Allegra S, Mercuri NB (2005). Activation of TRPV1 in the VTA excites dopaminergic neurons and increases chemical- and noxious-induced dopamine release in the nucleus accumbens. *Neuropsychopharmacology* **30**: 864–870.

Martin RS, Secchi RL, Sung E, Lemaire M, Bonhaus DW, Hedley LR *et al* (2003). Effects of cannabinoid receptor ligands on psychosis-relevant behavior models in the rat. *Psychopharmacology* **165**: 128–135.

McQueen DS, Bond SM, Smith PJ, Balali-Mood K, Smart D (2004). Cannabidiol lacks the vanilloid VR1-mediated vasorespiratory effects of capsaicin and anandamide in anaesthetised rats. *Eur J Pharmacol* **491**: 181–189.

Mezey E, Toth ZE, Cortright DN, Arzubi MK, Krause JE, Elde R *et al* (2000). Distribution of mRNA for vanilloid receptor subtype 1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. *Proc Natl Acad Sci USA* **97**: 3655–3660.

Moreira FA, Guimaraes FS (2005). Cannabidiol inhibits the hyperlocomotion induced by psychotomimetic drugs in mice. *Eur J Pharmacol* **512**: 199–205.

Parker LA, Burton P, Sorge RE, Yakiwchuk C, Mechoulam R (2004). Effect of low doses of delta9-tetrahydrocannabinol and

cannabidiol on the extinction of cocaine-induced and amphetamine-induced conditioned place preference learning in rats. *Psychopharmacology* **175**: 360–366.

Rakhshan F, Day TA, Blakely RD, Barker EL (2000). Carrier-mediated uptake of the endogenous cannabinoid anandamide in RBL-2H3 cells. *J Pharmacol Exp Ther* **292**: 960–967.

Ralph-Williams RJ, Lehmann-Masten V, Otero-Corchon V, Low MJ, Geyer MA (2002). Differential effects of direct and indirect dopamine agonists on prepulse inhibition: a study in D1 and D2 receptor knock-out mice. *J Neurosci* **22**: 9604–9611.

Ross RA (2003). Anandamide and vanilloid TRPV1 receptors. *Br J Pharmacol* **140**: 790–801.

Sakaue M, Ago Y, Baba A, Matsuda T (2003). The 5-HT1A receptor agonist MKC-242 reverses isolation rearing-induced deficits of prepulse inhibition in mice. *Psychopharmacology* **170**: 73–79.

Sasamura T, Sasaki M, Tohda C, Kuraishi Y (1998). Existence of capsaicin-sensitive glutamatergic terminals in rat hypothalamus. *Neuro Report* **9**: 2045–2048.

Smart D, Jonsson KO, Vandevoorde S, Lambert DM, Fowler CJ (2002). 'Entourage' effects of *N*-acyl ethanolamines at human vanilloid receptors. Comparison of effects upon anandamide-induced vanilloid receptor activation and upon anandamide metabolism. *Br J Pharmacol* **136**: 452–458.

Smit F, Bolier L, Cuijpers P (2004). Cannabis use and the risk of later schizophrenia: a review. *Addiction* **99**: 425–430.

Swerdlow NR, Geyer MA (1998). Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. *Schizophr Bull* **24**: 285–301.

Szallasi A, Di Marzo V (2000). New perspectives on enigmatic vanilloid receptors. *Trends Neurosci* **23**: 491–497.

Toth A, Boczan J, Kedei N, Lizanecz E, Bagi Z, Papp Z *et al* (2005). Expression and distribution of vanilloid receptor 1 (TRPV1) in the adult rat brain. *Brain Res Mol Brain Res* **135**: 162–168.

Van Der Stelt M, Di Marzo V (2004). Endovanilloids. putative endogenous ligands of transient receptor potential vanilloid 1 channels. *Eur J Biochem* **271**: 1827–1834.

Varty GB, Paulus MP, Braff DL, Geyer MA (2000). Environmental enrichment and isolation rearing in the rat: effects on locomotor behavior and startle response plasticity. *Biol Psychiatry* **47**: 864–873.

Watanabe K, Kayano Y, Matsunaga T, Yamamoto I, Yoshimura H (1996). Inhibition of anandamide amidase activity in mouse brain microsomes by cannabinoids. *Biol Pharm Bull* **19**: 1109–1111.

Wilson RI, Nicoll RA (2001). Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature* **410**: 588–592.

Yee BK, Chang DT, Feldon J (2004). The effects of dizocilpine and phencyclidine on prepulse inhibition of the acoustic startle reflex and on prepulse-elicited reactivity in C57BL6 mice. *Neuropsychopharmacology* **29**: 1865–1877.

Yue HY, Fujita T, Kawasaki Y, Kumamoto E (2004). AM404 enhances the spontaneous release of L-glutamate in a manner sensitive to capsazepine in adult rat substantia gelatinosa neurones. *Brain Res* **1018**: 283–287.

Zuardi AW, Morais SL, Guimaraes FS, Mechoulam R (1995). Antipsychotic effect of cannabidiol. *J Clin Psychiatry* **56**: 485–486.

Zuardi AW, Rodrigues JA, Cunha JM (1991). Effects of cannabidiol in animal models predictive of antipsychotic activity. *Psychopharmacology* **104**: 260–264.

Zuardi AW, Shirakawa I, Finkelfarb E, Karniol IG (1982). Action of cannabidiol on the anxiety and other effects produced by delta 9-THC in normal subjects. *Psychopharmacology* **76**: 245–250.

Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sorgard M, Di Marzo V *et al* (1999). Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature* **400**: 452–457.