

CB₁ cannabinoid receptors are involved in neuroprotection via NF- κ B inhibition

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We reported earlier that closed head injury (CHI) in mice causes a sharp elevation of brain 2-arachidonoylglycerol (2-AG) levels, and that exogenous 2-AG reduces brain edema, infarct volume and hippocampal death and improved clinical recovery after CHI. The beneficial effect of 2-AG was attenuated by SR141716A, a CB₁ cannabinoid receptor antagonist, albeit at relatively high doses. In the present study, we further explored the role of CB₁ receptors in mediating 2-AG neuroprotection. CB₁ receptor knockout mice (CB₁(-/-)) showed minor spontaneous recovery at 24 h after CHI, in contrast to the significant improvement in neurobehavioral function seen in wild-type (WT) mice. Moreover, administration of 2-AG did not improve neurological performance and edema formation in the CB₁(-/-) mice. In addition, 2-AG abolished the three- to four-fold increase of nuclear factor κ B (NF- κ B) transactivation, at 24 h after CHI in the WT mice, while it had no effect on NF- κ B in the CB₁(-/-) mice, which was as high as in the WT vehicle-treated mice. We thus propose that 2-AG exerts its neuroprotection after CHI, at least in part, via CB₁ receptor-mediated mechanisms that involve inhibition of intracellular inflammatory signaling pathways.

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Introduction

Traumatic brain injury leads to secondary damage that includes the release of harmful mediators (e.g. glutamate, reactive oxygen species (ROS), inflammatory cytokines). Protective mechanisms are also set in motion, and recently the endocannabinoid system was proposed to be neuroprotective (van der Stelt *et al*, 2002; Mechoulam, 2002; Mechoulam *et al*, 2002; Grundy *et al*, 2001). Cultured rat hippocampal neurons and cerebral cortical neurons are protected from excitotoxicity or ischemia by cannabinoid receptor agonists (Shen and Thayer, 1998; Sinor *et al*, 2000). In addition, N-methyl-D-aspartate-induced (NMDA) Ca²⁺ flux is reduced by anandamide, while SR141716A, a cannabinoid receptor (CB₁) antagonist, counteracted this activity (Hampson *et al*, 1998). *In vivo* models

support these observations. Thus, WIN 55212 (a synthetic cannabinoid) reduced ischemic damage in rat brain (Nagayama *et al*, 1999) and Δ^9 -tetrahydrocannabinol (THC), the main psychoactive marijuana constituent, reduced neuronal injury in neonatal rats injected with the Na⁺/K⁺-ATPase inhibitor ouabain (van der Stelt *et al*, 2001a). Hansen *et al* (2002) proposed that N-acylethanolamines, particularly anandamide, are neuroprotective and, indeed, anandamide protected rat brain against ouabain-induced neuronal injury (van der Stelt *et al*, 2001b). Yet, in contrast, a recent report describes the neurotoxic effects of anandamide in rats, through mechanisms independent of the CB₁ receptor and probably mediated, at least in part, via the vanilloid VR1 receptor (Cernak *et al*, 2004). We have reported that the levels of the endocannabinoid 2-arachidonoylglycerol (2-AG) increased 10-fold within 4 h after closed head injury (CHI) in mice, and that synthetic 2-AG injected after CHI improved outcome (Panikashvili *et al*, 2001). The neuroprotective effect of 2-AG was attenuated by the CB₁ receptor antagonist SR141716A, albeit at relatively high doses. These findings suggest that the neuroprotective effect of 2-AG is apparently cannabinoid receptor mediated.

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The mechanisms of neuroprotection or neurotoxicity by cannabinoids are not yet clear. Some cannabinoids, for example, plant derived (THC), synthetic (WIN 55212) or endogenous (2-AG, anandamide), bind to the CB₁ receptor; others, such as cannabidiol (CBD), a marijuana constituent (Hampson *et al*, 1998), or the synthetic dexanabinol (Shohami and Mechoulam, 2000; Knoller *et al*, 2002) do not. Hence, the effects of cannabinoids could be derived from numerous mechanisms.

Nuclear factor κ B (NF- κ B), a key regulator of inflammatory response (Perkins, 2000; Karin and Ben-Neriah, 2000) is composed of homo- and heterodimers including p65 and p50. The p65 subunit contains a translocation domain in its C-terminal end. Inactive NF- κ B is retained in the cytosol, where its activity is tightly regulated by members of the I κ B family. Activation of the I κ B kinase by different proinflammatory signals (e.g. endotoxin, tumor necrosis factor- α (TNF- α), IL-1 β , oxidative stress; Zingarelli *et al*, 2003) leads to phosphorylation, ubiquitination, and degradation of I κ B (Malek *et al*, 2001; Tam and Sen, 2001). Nuclear factor κ B, thus released, translocates into the nucleus and activates various genes. Studies from our laboratory recently showed robust transactivation of NF- κ B at 24 h up to 8 days after CHI. Inhibition of this activation by melatonin was associated with significant improved outcome (Beni *et al*, 2004).

The present study explores the effect of 2-AG on NF- κ B transactivation after CHI in mice. To confirm the role of the CB₁ receptor in neuroprotection, the spontaneous and 2-AG-mediated neurobehavioral recovery and NF- κ B activation in CB₁ receptor knockout (CB₁(-/-)) mice were compared with those observed in wild-type (WT) mice.

Materials and methods

Animals

The study was performed according to the guidelines of the Institutional Animal Care Committee. (1) Male C57BL/6J mice (Harlan, Israel) weighing 32 to 35 g were used in this study. The animals were divided into groups, treated with 2-AG or vehicle as described further, and killed at different times after CHI. (2) CB₁(-/-) mice were kindly provided by Professor A Zimmer (Bonn, Germany). They were developed as described previously (Zimmer *et al*, 1999). To transfer the CB₁ tm1zim mutation from the MPI2 embryonic stem cell genetic background (129/sv) to a C57BL/6J genetic background, congenic mice were generated by breeding the mutant allele for 10 generations with C57BL/6J mice. Heterozygous mice were bred to yield WT, heterozygous and null mice.

Trauma Model

Mice were subjected to CHI under ether anesthesia, confirmed by loss of pupillary reflex, using a weight-drop

device that falls over the left hemisphere, as described elsewhere (Chen *et al*, 1996) and modified by Yatsiv *et al* (2002). In brief, after a sagittal scalp incision, mice were immobilized under a cylindrical calibrated weight drop device. A tipped teflon cone was placed 3 mm lateral to the midline and 1 mm caudal to the left coronal suture, and a metal rod (94 g) was dropped on the cone from a height of 11 to 14 cm (adjusted to body weight) to cause CHI. Sham-treated mice were anesthetized with ether, their scalps were incised, but trauma was not induced.

Evaluation of Functional Outcome

At 1 h after CHI, the functional status of the mice was evaluated according to a set of 10 neurobehavioral tasks (neurological severity score (NSS)) that test reflexes, alertness coordination, and motor abilities (Table 1; Beni-Adani *et al*, 2001). One point was awarded for absence of reflex or failure to perform a particular task. Hence, a score of 10 reflects maximal neurological impairment. Only mice with NSS >4 at 1 h after injury (NSS 1 h) were included in the study. Immediately after evaluation of NSS 1 h, mice were randomly assigned to vehicle or drug treatment (see below), and NSS was evaluated again at 24 h. The extent of spontaneous recovery was calculated as the difference between NSS 1 h and that at 24 h: Δ NSS = NSS (1 h) - NSS (24 h) and compared with that induced by 2-AG treatment.

Cerebral Edema

Cerebral edema was evaluated at 24 h after CHI (time for maximal edema, Chen *et al*, 1996) by determining the tissue water content in the injured brain, as previously described (Chen *et al*, 1996). The percentage of tissue water was calculated as

$$\%H_2O = [(wet\ weight - dry\ weight) / wet\ weight] \times 100.$$

Infarct Volume

At 24 h after CHI brains of WT and CB₁(-/-) mice were sliced to 2-mm thick slices using a brain mold. The slices

Table 1 Neurological severity score for head-injured mice

TASK	NSS
Presence of mono- or hemiparesis	1
Inability to walk on a 3-cm wide beam	1
Inability to walk on a 2-cm wide beam	1
Inability to walk on a 1-cm wide beam	1
Inability to balance on a 1-cm wide beam	1
Inability to balance on a round stick (0.5-cm wide)	1
Failure to exit a 30-cm diameter circle (for 2 mins)	1
Inability to walk straight	1
Loss of startle behavior	1
Loss of seeking behavior	1
Maximum total	10

One point is awarded for failure to perform a task. NSS at 1 h in the range of 8 to 10: severe CHI.

were placed in a 2% solution of 2,3,5-triphenyltetrazolium chloride (TTC) in PBS and photographed using Stereoscope Stemi SV11 (Zeiss, Germany) and digital photcamera Coolpix E990 (Nikon, Japan). Scion Image-Release Beta 4.0.2 program was used to quantify the infarct volume, to examine whether lacking CB₁ receptor will affect infarct volume.

Electrophoretic Mobility Shift Assay for Nuclear Factor κ B DNA Binding

Mice were decapitated 8 and 24 h after CHI or sham operation. Nuclear extracts were prepared as described previously (Beni *et al*, 2004). The injured tissue weighing approximately 100 mg was dissected on ice, transferred briefly into ice-cold 0.32 mol/L sucrose and incubated for 5 to 10 mins. Tissues were homogenized in ice-cold 1:4 (w:v) buffer A (0.5 mol/L sucrose; 10 mmol/L HEPES, pH 7.9; 1.5 mmol/L MgCl₂; 10% glycerol; 1 mmol/L EDTA) into which 1 mmol/L DDT, 1 mmol/L PMSF and protease inhibitor cocktail (1:25; Roche Diagnostics; Mannheim, Germany) were added before use. After 15-min incubation on ice, centrifugation at 10,000g for 5 mins at +4°C was performed. Supernatants were discarded and pellets were rewashed with 1:1 (v:v) buffer A and centrifuged at 10,000g for 5 min at +4°C. Supernatants were discarded and nuclear pellets were re-suspended in 1:1 (v:v) ice-cold buffer C (20 mmol/L HEPES, pH 7.9; 25% glycerol; 0.42 M NaCl; 0.2 mmol/L EDTA), to which 1 mmol/L DDT, 0.5 mmol/L PMSF were added freshly. After 30-min incubation on ice with frequent vortexing, the samples were centrifuged for 20 mins at 15,000g at +4°C, and the supernatants were stored at -80°C until used as nuclear extracts in the EMSA. The consensus sequence for NF- κ B was a double-stranded oligonucleotide (5'-AGT TGA GGG GAC TTT CCC AGG C-3'; 3'-TCA ACT CCC CTG AAA GGG TCC G-5'; Promega, E3291; Madison, WI). Oligonucleotides contained 5'-OH blunt ends that were labeled with [γ -³²P] (Perkin-Elmer Life Sciences) using T₄ polynucleotide kinase (Promega, M4101) according to the instructions of the manufacturer. The binding reaction mix, containing 10 mmol/L HEPES (pH 7.9), 60 mmol/L KCl, 10% glycerol, 2 μ g bovine serum albumin, 0.4 mmol/L DDT, 2 μ g poly(dI-dC), 10 μ g nuclear protein, and γ -³²P-labeled NF- κ B (30,000 cpm), was incubated in ice for 1 h. Specificity of the protein-DNA complexes was confirmed by incubation (30 mins) of nuclear extracts with 100-fold excess of unlabeled NF- κ B oligonucleotide before the respective γ -³²P-labeled probe was added. Supershift assay for NF- κ B entailed incubation (30 mins) of nuclear extracts with 3 μ l of anti-p65 and anti-p50 monoclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) before addition of the γ -³²P-labeled probe. DNA-protein complexes were resolved on a 5% polyacrylamide gel made up in 1 \times TGE (50 mmol/L Tris, 400 mmol/L glycine, 2 mmol/L EDTA) at 100 V for 95 mins. The gels were vacuum-dried and exposed to Kodak X-ray films at -80°C. Quantitative data were obtained using Bio-Rad Multi-Analyst (PC Version 1.1). Levels of NF- κ B were expressed as the relative optical densities against background within a gel.

Each experiment was repeated three to four times, and the data represent the mean of all measurements.

Drugs

2-Arachidonoylglycerol was synthesized in our laboratory according to published procedures (Mechoulam *et al*, 1995). Emulphor was obtained from Sigma Israel. 2-Arachidonoylglycerol was dissolved in anhydrous ethanol:emulphor: saline (1:1:18) and injected intraperitoneally at 100 μ l per 10 g body weight at a dose of 5 mg/kg.

Statistical Analysis

Values represent the mean \pm s.d. Statistical significance of differences between means was evaluated by the nonparametric Mann-Whitney test for NSS assessment, and by Student's *t*-test for brain water content, and OD measurement. Probability values (*P*) smaller than 0.05 were considered to be statistically significant.

Results

Spontaneous Recovery after Closed Head Injury in Wild-Type and CB₁ Receptor Deficient Mice

To assess the basal motor activity of CB₁(-/-) mice, the NSS was recorded for naive CB₁(-/-) and WT mice (*n* = 7 and 9, respectively). Neurological severity score was higher in the CB₁(-/-) than in the WT mice (1.79 \pm 0.58 versus 0.56 \pm 0.88, *P* = 0.008), indicating some basal deficits, as reported earlier (Zimmer *et al*, 1999). CB₁(-/-) and WT mice were then subjected to CHI and their neurobehavioral outcome was evaluated 1 and 24 h later. Owing to the basal difference in NSS, it is hard to claim that similar values of NSS at 1 h indicates similar severity of injury in both groups, rather, similar NSS 1 h may bias towards more severe injury in the WT. Yet, over the next 24 h the WT mice recovered significantly better than the CB₁(-/-) mice as depicted by their higher Δ NSS (1.89 \pm 0.78 versus 0.71 \pm 0.76, respectively, *P* = 0.0164) (Figure 1A). By expressing the recovery as a difference between the NSS at 1 h and that at 24 h, each mouse serves as its own control, and Δ NSS for the individual mice indeed reflects their post CHI recovery, which is independent of the pre-CHI deficits. Thus, it appears that the CB₁(-/-) mice are more susceptible to the secondary brain damage after CHI than WT mice, and their spontaneous recovery is slower.

Infarct Volume and Edema after Closed Head Injury in CB₁ Receptor Deficient Mice

Using TTC staining 24 h after CHI infarct volume was measured in WT and CB₁(-/-) mice. In spite of the difference in functional recovery, there was no difference in the infarct volumes between the groups

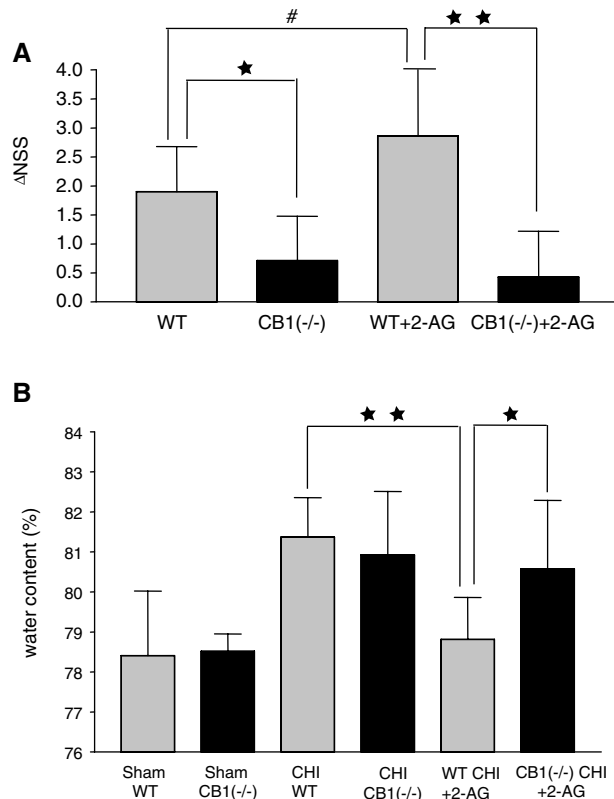


Figure 1 CB₁(-/-) mice display smaller spontaneous recovery after CHI as compared with WT mice, and do not respond to 2-AG therapy. **(A)** Motor function was assessed using the NSS; a neurobehavioral score that awards one point for failure to perform a task. The spontaneous recovery of the CB₁(-/-) ($n = 7$; filled bars) was significantly smaller than that of the WT ($n = 9$; gray bars). 2-Arachidonoylglycerol treatment, which increased Δ NSS in the WT, was not effective in the CB₁(-/-) ($n = 7$ /group). * $P < 0.05$ CB₁(-/-) versus WT. ** $P < 0.05$ CB₁(-/-) versus WT after 2-AG treatment. # $P < 0.05$ effect of 2-AG on WT. **(B)** Basal water content (sham mice) was similar in WT (gray bars) and CB₁(-/-) (filled bars) mice, and a similar increase occurred after CHI. However, treatment with 2-AG effectively reduced edema in the WT, but not in the CB₁(-/-) mice. * $P < 0.05$ WT treated with 2-AG versus CB₁(-/-) treated with 2-AG. ** $P < 0.05$ WT versus WT treated with 2-AG.

12.3 \pm 7.4 versus 15 \pm 10.6 respectively, $P = 0.51$. Similarly, basal water content in the cortex of nontraumatized CB₁(-/-) mice was similar to that of the WT. After CHI, significant water accumulation was found in both groups (Figure 1B), but edema was not statistically different between these groups (81.37 \pm 0.99 versus 80.92 \pm 1.59 %).

Effect of Exogenous 2-Arachidonoylglycerol on Neurological Severity Score and Edema in Wild-Type and CB1(-/-) Mice

We next addressed the question whether the protective effects of exogenous 2-AG, which were reported earlier, are mediated via the CB₁ receptor.

Wild-type and CB₁(-/-) mice were treated with 2-AG 1 h after CHI. 2-Arachidonoylglycerol significantly improved the neurobehavioral status of the WT, but not of the CB₁(-/-) mice (Δ NSS = 2.86 \pm 1.46 versus 0.43 \pm 0.79, respectively; $P = 0.0041$, Figure 1A). Moreover, treatment with 2-AG effectively reduced edema only in the WT mice (Figure 1B, 81.37 \pm 0.99, where water content was down to normal (78.81 \pm 1.046), but not in the CB₁(-/-), where it remained as high as in the nontreated mice (80.57 \pm 1.71, $P = 0.0308$ versus WT treated with 2-AG). It should be noted that basal water content, namely in sham-operated mice, in CB₁(-/-) mice was similar to that in the WT (78.52 \pm 0.43 versus 78.41 \pm 1.61).

Effect of 2-Arachidonoylglycerol on Nuclear Factor κ B Translocation

Since inflammation and oxidative stress are major components of the post-CHI responses, we decided to investigate the effect of 2-AG on a key transcription factor of these pathways. The pattern of NF- κ B-DNA binding of nuclear extract prepared 24 h after CHI is described in Figure 2A. Three bands were obtained and their specificity was proved by adding excess of unlabeled (cold) oligonucleotide NF- κ B and specific antibodies for the p65 and p50 subunits. Supershift assays show that the lower band is mostly composed of p50 and the upper band is p50-p65 heterodimer. Closed head injury in control mice (lanes 5 and 9) induced significant increase in NF- κ B-DNA binding as compared with sham (lane 10) (optical density 0.84 \pm 0.29 versus 0.27 \pm 0.15; Figure 2B). Treatment of WT mice with 2-AG (lanes 1, 3, 6, 8) completely abolished this increase (0.37 \pm 0.27; $P < 0.001$). We next investigated NF- κ B transactivation after CHI in the CB₁(-/-) mice, with and without 2-AG treatment. Figure 3 depicts the results of this study showing that, like in the WT, there is approximately three-fold increase of NF- κ B activation 24 h after CHI in CB₁(-/-) mice (lanes 2 and 9 versus 6). However, in contrast to WT mice, treatment with 2-AG in CB₁(-/-) was not effective (lanes 3, 5, 8 and 10), and did not reduce activity of this transcription factor (optical density 1.37 \pm 0.8 versus 1.53 \pm 0.62; Figure 3B).

Discussion

The present study extends our findings on the beneficial effect of 2-AG after CHI in mice, shows the role of the CB₁ receptor in mediating these effects and provides some mechanistic clue to its action. We reported earlier (Panikashvili *et al*, 2001) that 2-AG treatment decreased edema formation, hippocampal cell death, infarct volume and neurological dysfunction. The CB₁ antagonist SR141716A partly inhibited 2-AG protection, albeit at a relatively high dose

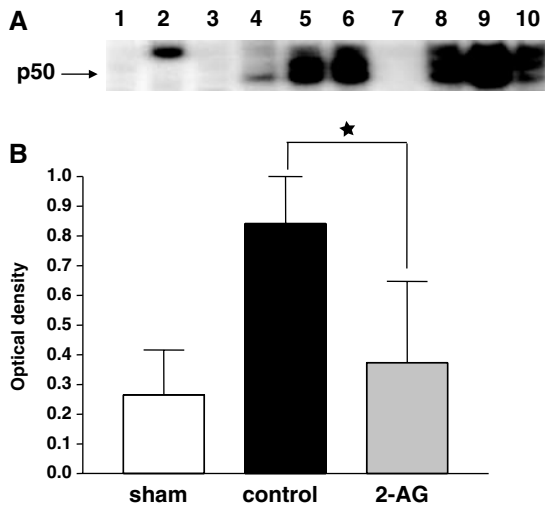


Figure 2 2-Arachidonoylglycerol inhibits NF- κ B nuclear translocation 24 h after CHI. **(A)** Nuclear factor κ B DNA binding was determined in brain nuclear extracts, using EMSA. Samples were isolated 24 h after CHI from control (vehicle-treated mice; lanes 5,9), from 2-AG-treated mice (lanes 1,3,6,8) and from sham-operated mice (lane 10); lane 7: competition with excess of unlabeled NF- κ B oligonucleotide. Supershift assay of a vehicle-treated sample pre-incubated with anti-p65 (lane 4) or anti-p50 (lane 2) antibodies (see text). **(B)** Quantitative data, expressed as relative optical densities, were obtained from the gel shown in **(A)**, using the Bio-Rad Multi-Analyst. Each experiment was repeated four to five times, and the data represent the mean \pm s.d. of all the measurements. * $P < 0.05$ versus control.

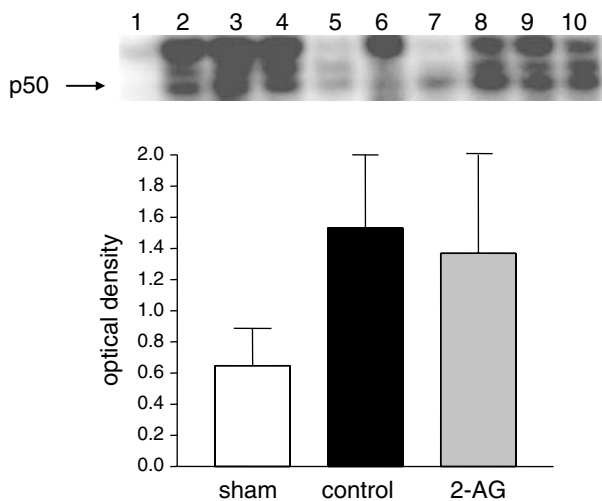


Figure 3 2-Arachidonoylglycerol does not abolish the increase in NF- κ B transactivation 24 h after CHI in CB₁(-/-) mice. **(A)** A pattern of EMSA similar to that shown in Figure 2 for the WT mice was found for the CB₁(-/-) control CHI mice (lanes 2,4,9). Treatment with 2-AG (lanes 3,5,8,10) had no effect on NF- κ B nuclear translocation. Anti p65 was added to mouse in lane 7, competition with protein excess is shown in lane 1, and sham mouse is shown in lane 6. **(B)** Quantitative data of Figure 3A, expressed as relative optical densities, calculated as described in Figure 2. Sham mice—empty bar ($n = 2$); control CHI-filled bar ($n = 4$) and 2-AG treated mice—gray bar ($n = 5$).

(20 mg/kg). Since the major brain cannabinoid receptor is CB₁, and the specificity of SR141716A as a pure CB₁ antagonist is controversial, we decided to use the CB₁ knockout mice to study the role of CB₁ receptor in neuroprotection. As reported by Zimmer *et al* (1999), we also found that the naive CB₁(-/-) mice display some motor deficits, unrelated to the trauma. As expected, after injury, their spontaneous recovery was extremely slow, as compared with the WT mice, suggesting that the endogenous cannabinoids play a role in the spontaneous recovery after CHI. In another experiment (preliminary data, not shown), NSS was evaluated at 3 and 7 days, and the same pattern was found, namely, Δ NSS of the CB₁(-/-) mice remained below 1 during the whole period, while that of the WT slowly, but consistently, increased. These findings agree with those of Parmentier-Batteur *et al* (2002), who showed increased severity of stroke in CB₁(-/-) mice. However, the present findings do not show greater infarct volume or edema in the CB₁(-/-), indicating that other endogenous mechanisms are probably involved. To further confirm the role of the CB₁ receptor in 2-AG neuroprotection, WT and CB₁(-/-) mice were treated with exogenous 2-AG. As expected, no beneficial effects, neither on neurobehavior nor on edema formation, were noted in the CB₁(-/-) mice, in contrast to a significant effect on the WT, confirming our earlier reports (Panikashvili *et al*, 2001).

Proinflammatory cytokines play a crucial role in traumatic brain injury. Tumor necrosis factor- α is released early (within 1 to 4 h) after CHI (Shohami *et al*, 1997; Stover *et al*, 2000) and acts on specific receptors. On binding, the cytosolic portions of both TNF receptors recruit multiple intracellular adapter proteins that activate the transcription factor NF- κ B, starting with hydrolysis of the inhibitory protein I κ B that allows the p65/p50 complex to translocate to the nucleus and to regulate the expression of various (Baeuerle and Henkel, 1994). We have recently reported that inhibition of NF- κ B transactivation after CHI (at 1 to 8 days) is associated with better functional outcome (Beni *et al*, 2004), and therefore decided to investigate the effect of 2-AG on NF- κ B activation after CHI. Indeed, treatment with 2-AG completely abolished the robust activation of this transcription factor, which is a key event in the proinflammatory signaling after CHI.

Closed head injury-induced activation of NF- κ B in the CB₁(-/-) mice was three to four fold higher than in the respective noninjured (sham) CB₁(-/-) mice, similar to that observed in the WT. This suggests that the endogenous 2-AG does not affect the inflammatory signaling. However, in response to exogenous 2-AG, WT and CB₁(-/-) mice differed dramatically, and the latter did not respond at all to 2-AG treatment. In addition to their antiinflammatory activities, cannabinoids act also as antioxidants. Cannabidiol is a potent antioxidative agent (Hampson *et al*, 1998) and the nonpsychotropic

synthetic cannabinoid, dexanabinol (HU-211), has also antioxidative properties (Shohami and Mechoulam, 2000). 2-Arachidonoylglycerol was also shown to suppress formation of ROS and TNF- α by murine macrophages *in vitro* after stimulation with lipopolysaccharide (Gallily *et al*, 2000). The antioxidant properties of 2-AG may well add to its profile as neuroprotectant and inhibitor of NF- κ B transactivation. Oxidative stress is one of the major components in the pathophysiology of traumatic brain injury (Lewen *et al*, 2000), and ample evidence suggests that ROS also regulate signal transduction pathways such as the NF- κ B and AP-1 (Vollgraf *et al*, 1999). Thus, taken together, anti-inflammatory and antioxidant properties of 2-AG may either add or synergize to enhance its activity as a neuroprotective agent.

Cannabinoids produce a variety of neurobehavioral effects, and a major focus of cannabinoid research has been the substantiation of the assumption that the pharmacological actions of cannabinoids are receptor-mediated. Indeed, the CB₁(-/-) mice showed spontaneous phenotypes, including hypoactivity, reduced locomotion and rearing, supraspinal hypoalgesia, increased mortality (Zimmer *et al*, 1999), spontaneous reduction in feeding behavior (Di Marzo *et al*, 2001), changes in male hormone balance (Paria *et al*, 2001), and suckling behavior within 1 day of birth (Fride *et al* (2001). For the most part, results observed in mice treated with selective CB₁ receptor antagonists mimic the findings observed in the transgenic animals. However, developmental changes may occur to compensate for the lack of CB₁ receptors, as has been suggested from studies of neuropeptide expression (Steiner *et al*, 1999). Our current findings fit into this body of evidence regarding the importance of the endocannabinoid system, acting via the CB₁ receptor, in brain function under physiological and pathological conditions. Recently, van der Stelt *et al* (2002) and Mechoulam (2002) discussed the role of the endocannabinoid system as a general endogenous protection system. The pharmacological picture is further complicated by the fact that there seems to be species differences. While we found 2-AG production to be enhanced in mice after CHI, Panikashvili *et al* (2001) and Sugiura *et al* (2000) saw elevation in 2-AG level in picrotoxin-administered rat brain; Hansen *et al* (2001), in contrast, found anandamide and not 2-AG enhancement in rats after TBI.

Several pharmacological agents have been described to inhibit NF- κ B at one or multiple activation steps of the signaling pathway. These agents include proteasome inhibitors, glucocorticoids (such as dexamethasone), nonsteroidal anti-inflammatory drugs and anti-inflammatory cytokines (Zingarelli *et al*, 2003). Cannabinoids were found to be very effective in different models of inflammation. In the 1970s, Sofia *et al* (1973a,b, 1974) showed robust anti-inflammatory effects of crude marijuana

extract, of the active marijuana constituent THC, as well as of the marijuana nonpsychoactive constituents CBD and cannabinol (CBN) in paw edema inflammation model in rats. Some of these effects were later shown to be CB₁ or CB₂ receptors mediated (Clayton *et al*, 2002; Hanus *et al*, 1999). Cannabinol and 2-AG were shown to inhibit IL-2 expression in activated thymocytes through inhibition of NF- κ B (Herring and Kaminski, 1999; Ouyang *et al*, 1998). Yet, the molecular mechanisms of all these beneficial effects remained unclear. The complexity and controversy in the field of endocannabinoids is further shown in a recent report on the 'dark side' of anandamide that describes its toxic effects, both *in vitro* and *in vivo*, in rats (Cernak *et al*, 2004). This study suggests that mechanisms independent of the CB₁ receptor, probably vanilloid receptor VR1-mediated, are involved in anandamide's neurotoxicity.

In conclusion, we report for the first time that the endocannabinoid 2-AG exerts neuroprotection after traumatic brain injury, at least in part, by inhibition of NF- κ B transactivation through CB₁ receptors. We suggest to further study drugs of similar pharmacological profile as novel candidates for treatment of traumatic brain injury.

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