Effects of CB_1 cannabinoid receptor activation on cerebellar granule cell nitric oxide synthase activity

Cecilia J. Hillard*, Shanmugam Muthian, Christopher S. Kearn

Department of Pharmacology and Toxicology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226 USA

Received 30 July 1999; received in revised form 9 September 1999

Abstract Cerebellar granule cells (CGCs) express the CB_1 subtype of cannabinoid receptor. CB_1 receptor agonists Win 55212-2, CP55940 and HU210 inhibit KCl-induced activation of nitric oxide synthase (NOS) in CGCs. Win 55212-2 has no effect on either basal NOS activity or on activation by *N*-methyl-D-aspartate and its effect is abolished by pre-treatment of the cells with pertussis toxin. The CB_1 receptor antagonist/inverse agonist SR141716A both reverses the effects of Win 55212-2 and produces an increase in NOS activity that is additive with KCl. These results support the hypothesis that activation of the CB_1 receptor in CGCs results in a decreased influx of calcium in response to membrane depolarization, resulting in a decreased activation of neuronal NOS.

Key words: Calcium channel; Inverse agonist; SR141716A; Win 55212-2

1. Introduction

The psychoactive cannabinoid Δ^9-tetrahydrocannabinol (THC) [1] affects the cellular function as a result of binding to a G protein-coupled receptor [2]. This receptor, termed CB_1 [3], is also activated by synthetic analogs of THC, including CP55940 [2] and HU210 [4], by a series of aminoalkyl indoles, including Win 55212-2 [5], and by the endocannabinoids *N*-arachidonylethanolamine (AEA) [6] and 2-arachidonylglycerol [7,8]. SR141716A has recently been identified as an antagonist/inverse agonist of the CB_1 receptor [9,10].

The CB_1 receptor is found in high density in many regions of the brain, including the molecular layer of the cerebellum [11]. Cerebellar granule cells (CGCs) in primary culture express the CB_1 receptor and respond to CB_1 receptor agonists with a decrease in adenylyl cyclase activity and activation of GTPase activity [12]. In addition, CB_1 receptor activation in CGCs results in inhibition of the opening of voltage-operated calcium channels [13] in a manner very similar to CB_1 inhibition of N-type calcium channels in NG108 cells [14]. One possible consequence of a reduction in calcium influx is decreased activation of various calcium dependent intracellular processes, including the synthesis of nitric oxide (NO) by neuronal NO synthase (nNOS). CGCs express high levels of nNOS [15–17], which is activated in response to increased intracellular calcium. This can be achieved through either depolarization-induced opening of voltage-operated calcium channels or activation of *N*-methyl-D-aspartate (NMDA) glutamatergic receptors [18]. The purpose of the studies described herein was to test the hypothesis that CB_1 receptor agonists inhibit depolarization-induced activation of NOS as a result of inhibition of calcium influx through voltage-operated calcium channels.

2. Materials and methods

2.1. CGC primary culture

CGCs were prepared from 6–8 day old rat pups of either sex exactly as described previously [19]. The cells were plated onto six well plates coated with poly-D-lysine and were used for an assay at 7–10 days in vitro.

2.2. [^{3}H]SR141716A binding assay

CGCs were grown in 12 well dishes and were washed with 3×0.5 ml of warm HEPES buffer (154 mM NaCl, 5.6 mM KCl, 3.6 mM $NaHCO_3$, 5.6 mM glucose, 1.3 mM $CaCl_2$ and 10 mM HEPES, pH 7.35) containing 0.1% fatty acid free bovine serum albumin (BSA). Cells were incubated with [^{3}H]SR141716A (0.05–2.75 nM) in 0.5 ml of HEPES/BSA buffer for 30 min at 37°C. After removal of the incubation buffer, cells were transferred to ice and washed three times with 1 ml of ice cold buffer containing BSA. After washing, the cells were scraped into 0.5 ml of water and counted. Non-specific binding was determined in wells containing 10 μM THC. The resulting saturation isotherms were fit to the single site binding equation using non-linear regression (GraphPad Prism Software, San Diego, CA, USA).

2.3. Assay of NOS activity

NOS activity was measured using the conversion of [^{3}H]arginine to [^{3}H]citrulline using a modification [20] of the method of Bredt and Snyder [17]. Granule cells were pre-incubated with the cannabinoids or vehicle and [^{3}H]arginine (3 μCi) in HEPES/BSA buffer for 5 min at 37°C. The stimulator (i.e. KCl or NMDA) or buffer alone was added and the incubation was continued for 5 min. In some experiments, 1 μCi $^{45}CaCl_2$ was added at the same time as the stimulator. After 5 min, the buffer was removed and the cells were washed three times with 3 ml of cold buffer. The washed cells were scraped in 1 ml of 0.3 mM $HClO_4$, the lysate was centrifuged and the supernatant was neutralized with K_2CO_3. An aliquot of the supernatant was counted for both ^{3}H (to assess arginine uptake) and ^{45}Ca (to assess calcium entry during stimulation). A second aliquot of the supernatant was placed onto columns containing Dowex AG 50W-X8 cation exchange resin (sodium form). The flow through and eluate following the addition of 2 ml of water were combined and counts per minute (cpm) were determined. Studies using [^{14}C]citrulline and [^{3}H]arginine standards demonstrate that the columns retain over 98% of added arginine and less than 7% of added citrulline.

2.4. Measurement of AEA synthesis by CGCs

AEA synthesis was measured using a precursor pre-loading method similar to that described previously [21]. CGCs were pre-loaded with [^{3}H]arachidonic acid (AA) for 20–24 h and were then washed and incubated with either buffer alone or with 35 mM KCl. After the incubation, the cells and buffer were extracted with chloroform:methanol (1:2) using established methods [22] and were separated using reverse phase high pressure liquid chromatography (rpHPLC) using a Nucleosil C-18 column (5 μ, 4.6×250 mm). Solvent A (water) and solvent B (acetonitrile containing 0.1% glacial acetic acid) were varied from 50% A in B to 100% B over 40 min at a flow rate of 1 ml/min.

*Corresponding author. Fax: (1) (414) 456-6545.
E-mail: chillard@mcw.edu

PII: S0014-5793(99)01253-3

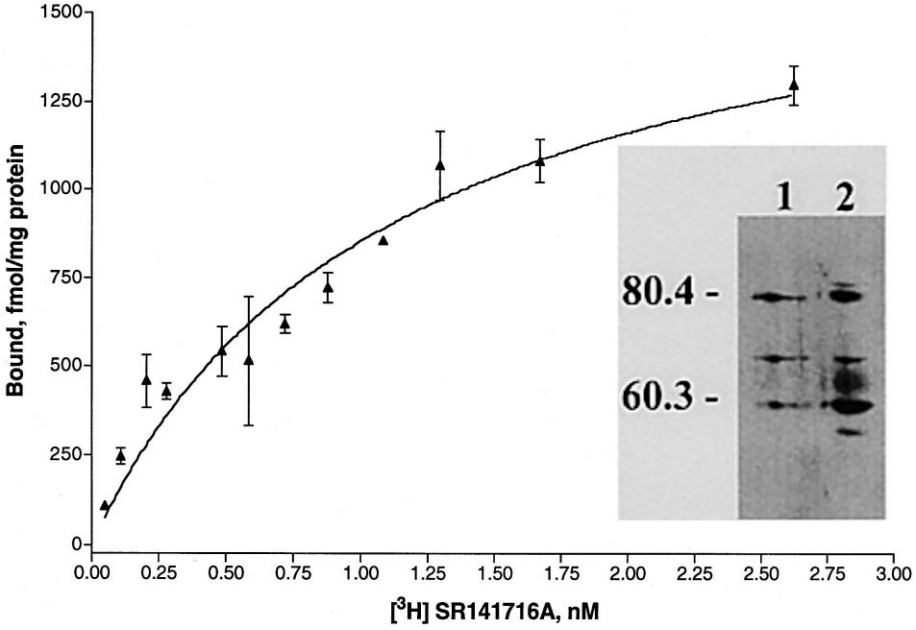

Fig. 1. CGC membranes bind [^{3}H]SR141716A in a saturable manner. CGCs (8 days in vitro) were incubated with [^{3}H]SR141716A for 30 min at 37°C, washed, lysed and the lysates were counted. Non-specific binding was defined using 10 μM THC and has been subtracted. Data shown are the mean of two separate experiments. The line is the non-linear regression fit of the data to the single site binding equation. Inset: Western blot of CGC membranes (lane 1) and rat forebrain membranes (lane 2) probed using an affinity-purified polyclonal antibody to the CB_1 receptor (provided by Dr Ken Mackie). Each lane contained 10 μg of membrane protein. Numbers on the left represent the molecular weight.

The column eluate was collected in 0.2 ml fractions and the radioactivity determined.

2.5. Materials

[^{3}H]Arginine (specific activity 38.5 Ci/mmol), $^{45}CaCl_2$ (22 mCi/mg), [^{3}H]AA (185 Ci/mmol) and [^{3}H]AEA (223 Ci/mmol) were purchased from Du Pont New England Nuclear (Boston, MA, USA). [^{3}H]SR141716A (specific activity 40–50 Ci/mmol) was purchased from Amersham Radiochemicals (Arlington Heights, IL, USA). Win 55212-2 was purchased from RBI Chemicals (Natick, MA, USA). CP55940 was provided by Pfizer Central Research (Groton, CT, USA), HU210 was a gift of Dr Raphael Mechoulam (Hebrew University, Israel), SR141716A was provided by Sanofi Researche (Montpellier, France) and Δ^9-THC was obtained from the National Institute on Drug Abuse (Rockville, MD, USA). All other drugs and chemicals were of the highest grade possible and were purchased from standard commercial sources.

2.6. Statistics

Treatment groups were compared using one way analysis of variance followed by *t*-tests. A P value of less than 0.05 was used to determine statistical significance.

3. Results

3.1. CGCs in primary culture express the CB_1 receptor

As has been shown previously [12], CGCs at 7–10 days in primary culture express the CB_1 subtype of the cannabinoid receptor. Membranes prepared from CGCs were positive for the CB_1 receptor in Western blot analysis (Fig. 1). CGCs exhibit saturable binding of the selective CB_1 receptor antagonist/inverse agonist, [^{3}H]SR141716A (Fig. 1). The saturation isotherms were fit to the single site binding equation and the binding parameters were determined (K_D 1.1 ± 0.3 nM and B_{max} 1.8 pmol/mg protein).

3.2. Depolarization-induced activation of NOS is reduced by co-incubation with CB_1 receptor agonists

Both KCl and NMDA increased the conversion of [^{3}H]arginine to [^{3}H]citrulline (Table 1A). The effects of both stimuli were concentration-related, with maximal increases occurring at 35 mM KCl and 100 μM NMDA. These agents also produced increases in ^{45}Ca influx during a 5 min incubation, although these increases did not reach statistical significance.

Incubation of the CGCs with the CB_1 receptor agonist Win 55212-2 resulted in attenuation of KCl-induced NOS activation and ^{45}Ca influx (Fig. 2). The IC_{50} value for Win 55212-2 inhibition of NOS activity was 1.88 μM (95% confidence interval 1.2–2.9 μM) and the maximum inhibition was 66 ± 6%. The IC_{50} value for Win 55212-2 inhibition of ^{45}Ca was 0.90 μM (95% confidence interval 0.77–1.05 μM) and the maximum inhibition was 69 ± 2%. At a concentration of 10 μM, Win 55212-2 had no effect on the basal NOS activity or NOS activation by NMDA and produced a slight decrease in ^{45}Ca influx in response to NMDA that did not reach statistical significance (Table 1B).

Two other CB_1 receptor agonists, CP55940 and HU210, also attenuated depolarization-induced activation of NOS and influx of calcium in CGCs. HU210 inhibited NOS activity

Table 1A
Activation of NOS and calcium influx in CGCs

Stimulus	Fractional conversion to citrulline	Calcium influx (cpm)
Basal	0.142 ± 0.01	114 ± 51
KCl (35 mM)	0.248 ± 0.01*	213 ± 75
NMDA (100 μM)	0.191 ± 0.01*	294 ± 46

Table 1B
Effects of Win 55212-2 on NOS activity and calcium influx in CGCs

Stimulus	Fractional conversion to citrulline		Calcium influx (cpm)	
	Control	Win 55212-2 (10 μM)	Control	Win 55212-2 (10 μM)
Basal	0.138 ± 0.01	0.132 ± 0.01	88 ± 24	80 ± 26
100 μM NMDA	0.181 ± 0.01	0.174 ± 0.02	304 ± 60*	270 ± 48*

CGCs were maintained in culture for 8–12 days and were incubated with [^{3}H]arginine for 5 min prior to the addition of ^{45}Ca and either buffer ('Basal'), KCl or NMDA. The incubation was allowed to proceed for 5 additional min at which time the cells were washed and cell lysates were analyzed for [^{3}H]citrulline, [^{3}H]arginine or ^{45}Ca as described in Section 2. Data are shown ± S.E.M. Each value is the mean of 4–5 experiments carried out in duplicate.
*Significantly different from basal activity or calcium influx at $P < 0.05$ using Dunnett's t-test.

with an IC_{50} value of 0.52 nM (95% confidence interval 0.41–0.66 nM) and a maximal inhibition of 78 ± 4.2%. CP55940 inhibited NOS activity with an IC_{50} value of 7.3 nM (95% confidence interval 3.7–14 nM) and a maximal inhibition of 79 ± 13%. The two agonists also inhibited ^{45}Ca influx. At 3 nM HU210, KCl-induced ^{45}Ca influx was reduced by 85.5 ± 14.5% ($n = 3$) and at 10 nM CP55940, KCl-induced ^{45}Ca influx was reduced by 100% ($n = 2$).

3.3. SR141716A increases NOS activity in CGCs

SR141716A is a CB_1 receptor antagonist [23,24]. At a concentration of 100 nM, SR141716A produced a significant increase in the formation of [^{3}H]citrulline from [^{3}H]arginine, an increase that was approximately equal to the increase produced by 35 mM KCl (Fig. 3A). When SR141716A was added together with KCl, the effects of the two compounds were additive. When SR141716A was added together with Win 55212-2 and KCl, NOS activity was equal to the activity seen in the presence of KCl alone. That is, both SR141716A activation and Win 55212-2 inhibition were reversed. At a concentration of 300 nM, SR141716A doubled the amount of ^{45}Ca influx compared to a vehicle control (data not shown). The EC_{50} value for SR141716A was 15 nM (95% confidence interval 0.6–367 nM).

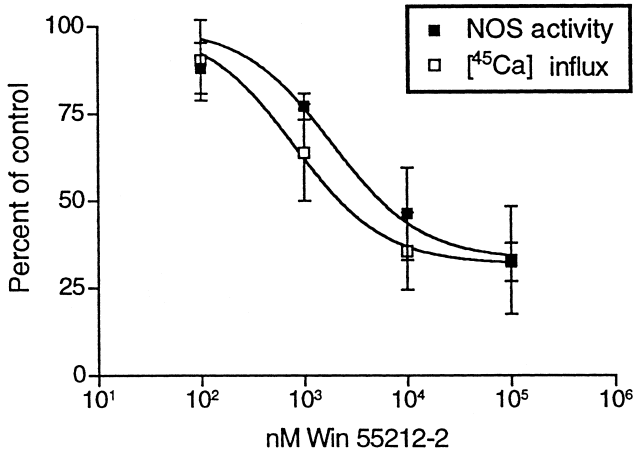

Fig. 2. Win 55212-2 inhibits both KCl-induced NOS activity and calcium influx in CGCs. CGCs (8 days in vitro) were pre-incubated with both Win 55212-2 (or DMSO vehicle) and [^{3}H]arginine for 5 min at 37°C. KCl (final concentration 35 mM) was added along with ^{45}Ca and the incubation was continued for 5 min. NOS activity was determined from the conversion of [^{3}H]arginine to [^{3}H]citrulline and ^{45}Ca influx was determined in the washed cell lysates. Shown are the mean data from four experiments, vertical lines represent S.E.M. Lines are the best fit of the data to the sigmoidal concentration-response curve. Control values were determined in cells pre-treated with DMSO.

The effects of SR141716A could be due to inhibition of the actions of an endogenous CB_1 agonist that is released when the cells are depolarized by KCl. To test this possibility, CGCs were pre-loaded with [^{3}H]AA for 24 h, then incubated with either buffer alone or buffer containing 35 mM KCl. After the incubation, cellular and media lipids were extracted and the radiolabelled species separated using rpHPLC. No radiolabelled species that co-migrated with [^{3}H]AEA were detected in either the buffer (data not shown) or KCl-treated (Fig. 4) CGCs.

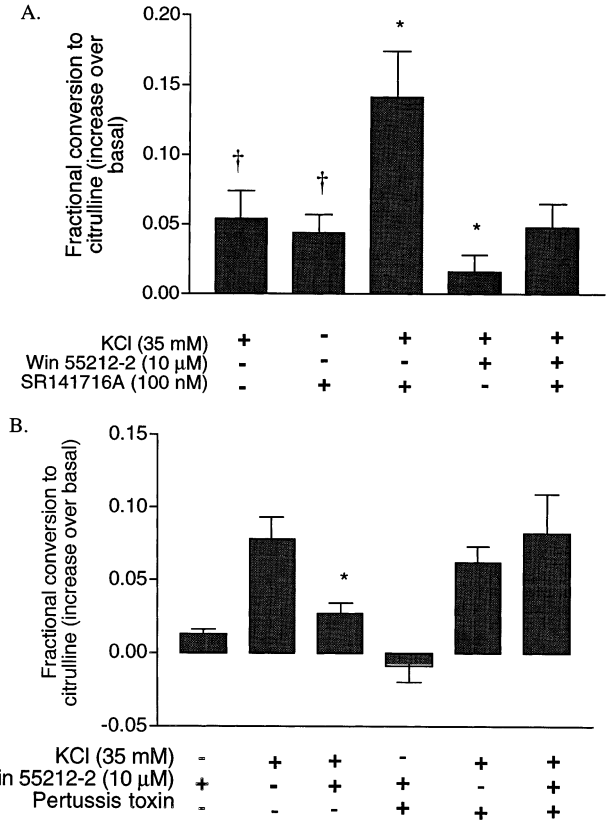

Fig. 3. A: CGCs were pre-incubated with either Win 55212-2, SR141716A, their combination or DMSO vehicle and [^{3}H]arginine for 5 min at 37°C. B: Cells were pre-treated with pertussis toxin (0.1 μg/well) for 24 h, followed by washing and pre-treatment with Win 55212-2 or DMSO and [^{3}H]arginine for 5 min at 37°C. In both, KCl (final concentration 35 mM) was added and the incubation was continued for 5 min. NOS activity was determined from the conversion of [^{3}H]arginine to [^{3}H]citrulline. NOS activity in the absence of KCl has been subtracted. Each bar represents the mean of three experiments, lines represent S.E.M. * indicates significantly different from KCl-treated cells pre-incubated with DMSO with $P < 0.05$. † indicates significantly increased over basal activity.

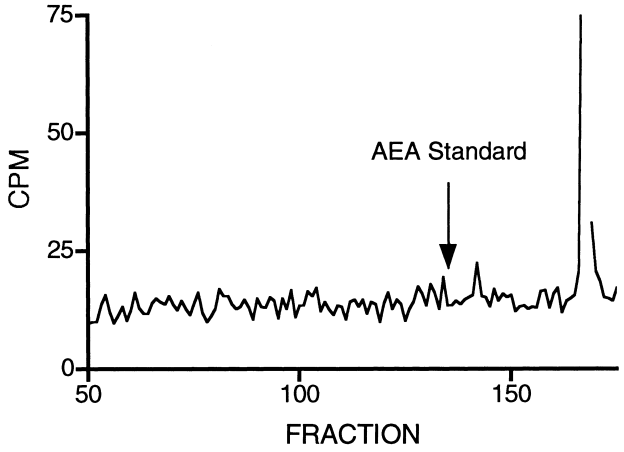

Fig. 4. Depolarization of CGCs does not affect the concentration of [^{3}H]AEA in the cultures. CGCs were pre-incubated with [^{3}H]AA for 20 h prior to stimulation with buffer containing 35 mM KCl. Following the stimulation, lipids were extracted from the cells and media and were separated using rpHPLC. An arrow indicates the fraction at which standard [^{3}H]AEA was detected. Shown is a representative from three similar experiments.

3.4. Reversal of the effects of Win 55212-2 by pertussis toxin

CGCs were pre-treated with pertussis toxin (0.1 μg/well) for 24 h. Control cells received media alone. Pertussis toxin pretreatment had no significant effect on NOS activation by 35 mM KCl but reversed Win 55212-2 inhibition of KCl-stimulated NOS activity (Fig. 3B).

4. Discussion

In agreement with the work of others [12,25,26], we have found that CGCs express a functional CB_1 receptor in primary culture. Activation of the CB_1 receptor in CGCs by the synthetic cannabinoid Win 55212-2 results in decreased KCl-mediated activation of NOS. In light of the well documented ability of CB_1 receptor agonists to reduce the calcium current through voltage-operated calcium channels [14,27,28], it is our hypothesis that CB_1 attenuation of NOS activity occurs as a result of inhibition of the influx of calcium in response to KCl. In support of this hypothesis, neither basal NOS activity (i.e. unstimulated) nor NOS activation by NMDA are affected by Win 55212-2, suggesting that inhibition occurs only when activator calcium enters the cells via VOCCs. In addition, Win 55212-2 inhibits ^{45}Ca influx into CGCs in response to KCl but not NMDA. The latter finding is in disagreement with the data of Hampson and coworkers who found that AEA, via the CB_1 receptor, reduced the influx of calcium into cerebellar slices [29]. Taken together, these data suggest that activation of the CB_1 receptor of CGCs reduces the synthesis of NO in response to depolarization via inhibition of calcium entry through VOCCs.

Win 55212-2 inhibition of KCl-stimulated NOS activity is mimicked by two other potent CB_1 agonists, CP55940 and HU210. The potency order of the agonists (HU210 > CP55940 > Win 55212-2) is consistent with other studies [30]. However, Win 55212-2 is somewhat less potent in this assay than in other assays of CB_1 receptor activation. For example, Win 55212-2 inhibits adenylyl cyclase in CGCs with an IC_{50} value of 0.1 μM [12], a concentration 10-fold lower than the IC_{50} values found in the present study. One possible explanation for the difference is that the present incubations were carried out with BSA in the buffer as a dispersing agent for the cannabinoids, while the earlier study used only DMSO as a vehicle [12].

SR141716A activated NOS and increased the influx of extracellular calcium, effects that were reversed by Win 55212-2. Since the CB_1 receptor is the only known site of action of low concentrations of SR141716A, the most likely explanation for these data is that SR141716A reverses or inhibits tonic activation of the CB_1 receptor. Tonic activation of the CB_1 receptor could either be due to the presence of an endogenous CB_1 agonist, such as AEA, or the result of constitutive activity of the CB_1 receptor [31]. Our evidence does not support the first possibility as 35 mM KCl did not increase the synthesis of AEA in CGCs. In support of the second possibility, SR141716A has been shown in several studies to act as an inverse agonist of the CB_1 receptor [9,32]. Inverse agonists are defined as ligands that occupy the ligand binding site of a receptor and decrease receptor coupling to G proteins [33,34]. As a result, inverse agonists can produce cellular changes that are opposite to those of agonists. Our data suggest that SR141716A is acting as an inverse agonist of the CB_1 receptor in CGCs and thereby imply that the CB_1 receptor functions as a brake on the activation of NOS in the absence of agonist. However, we cannot completely rule out either functional antagonism between Win 55212-2 and SR141716A that is not mediated completely by the CB_1 receptor or SR141716A antagonism of an endogenous CB_1 ligand that is not AEA.

Granule cell axons in vivo form the parallel fibers of the molecular layer of the cerebellum. The target of the parallel fibers are Purkinje cells, the major outflow cell of the cerebellum. The parallel fiber-Purkinje cell synapse is glutamatergic and has been studied as a model of synaptic plasticity. A recent report by Levenes and coworkers demonstrates that Win 55212-2, acting via the CB_1 receptor, inhibits glutamatergic transmission at parallel fiber-Purkinje cell synapses via a pre-synaptic effect [35]. The most likely mechanism for this effect is inhibition of pre-synaptic VOCCs, resulting in a decrease in the probability of glutamate release. Win 55212-2 also reduces the induction of long term depression at this synapse via a pre-synaptic effect. The authors of that study suggest that inhibition of NO synthesis by Win 55212-2 may play a role in this physiologic effect [35]. Our results are consistent with this suggestion.

There have been several other studies of the interactions of the cannabinoids with NOS which suggest that the actions of the cannabinoids on NOS may vary in different cell types. The cannabinoids anandamide and CP55940 have been shown to increase NO production by human monocytes and invertebrate immunocytes [36]. This effect is blocked by SR141716A, supporting a role of the CB_1 receptor in this action. Interestingly, a homologue of the CB_1 receptor has recently been identified in the leech central nervous system that also regulates NOS activity [37]. The synthesis of NO by NOS in human endothelial cells is also increased by anandamide [38], possibly due to CB_1-mediated increase in the release of intracellular calcium [39].

In summary, these data suggest that the neuronal CB_1 receptor plays a role in the regulation of NO synthesis. In light of the data that SR141716A increases NOS activity, we suggest that the CB_1 receptor is constitutively active and serves as

a brake on calcium entry in response to depolarization. As a result, CB_1 receptor agonists can further reduce NOS activity while the cell itself, via changes in the synthesis or activity of CB_1 receptors themselves, can increase (i.e. disinhibit) NOS activity. In light of the widespread role of NO as a modulatory agent in the brain, it is likely that NOS inhibition plays a role in the overall effects of cannabinoids on the brain function.

Acknowledgements: This work was supported by USPHS Grant DA08098. The authors are grateful for technical help from Bruce Peltier and Marcie Greenberg. The CB_1 antibody was generously provided by Dr Ken Mackie (University of Washington). A preliminary report of these findings was presented at the 1995 Experimental Biology meeting.

References

[1] Gaoni, Y. and Mechoulam, R. (1964) J. Am. Chem. Soc. 86, 1646–1647.
[2] Devane, W.A., Dysarz, F.A., Johnson, M.R.L.S.M. and Howlett, A.C. (1988) Mol. Pharmacol. 34, 606–613.
[3] Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C. and Bonner, T.I. (1990) Nature 346, 561–564.
[4] Devane, W., Breuer, A., Sheskin, T., Jarbe, T., Eisen, M. and Mechoulam, R. (1992) J. Med. Chem. 35, 2065–2069.
[5] Kuster, J., Stevenson, J., Ward, S., D'Ambra, T. and Haycock, D. (1993) J. Pharmacol. Exp. Ther. 264, 1352–1363.
[6] Devane, W.A. et al. (1992) Science 258, 1946–1949.
[7] Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., Yamashita, A. and Waku, K. (1995) Biochem. Biophys. Res. Commun. 215, 89–92.
[8] Mechoulam, R. et al. (1995) Biochem. Pharmacol. 50, 83–90.
[9] Bouaboula, M. et al. (1997) J. Biol. Chem. 272, 22330–22339.
[10] Rinaldi-Carmona, M. et al. (1995) Life Sci. 56, 1941–1947.
[11] Herkenham, M., Lynn, A.B., Little, M.D., Johnson, M.R., Melvin, L.S., DeCosta, B.R. and Rice, K.C. (1990) Proc. Natl. Acad. Sci. USA 87, 1932–1936.
[12] Pacheco, M.A., Ward, S.J. and Childers, S.R. (1993) Brain Res. 603, 102–110.
[13] Nogueron, I., Porgilsson, B. and Hillard, C.J. (1998) FASEB J. 12, A152.
[14] Mackie, K. and Hille, B. (1992) Proc. Natl. Acad. Sci. USA 89, 3825–3829.
[15] Southam, E., Morris, R. and Garthwaite, J. (1992) Neurosci. Lett. 137, 241–244.
[16] Fostermann, U., Gorsky, L.D., Pollock, J.S., Schmidt, H.H.H.W., Heller, M. and Murad, F. (1990) Biochem. Biophys. Res. Commun. 168, 727–732.
[17] Bredt, D.S., Hwang, P.M. and Snyder, S.H. (1990) Nature 347, 768–770.
[18] Garthwaite, J., Charles, S.L. and Chess-Williams, R. (1988) Nature 336, 385–388.
[19] Hillard, C.J., Edgemond, W.S., Jarrahian, A. and Campbell, W.B. (1997) J. Neurochem. 69, 631–638.
[20] Kiedrowski, L., Costa, E. and Wroblewski, J.T. (1992) J. Neurochem. 58, 335–341.
[21] Di Marzo, V., Fontana, A., Cadas, H., Schinelli, S., Cimino, G., Schwartz, J.C. and Piomelli, D. (1994) Nature 372, 686–691.
[22] Bligh, E. and Dyer, W. (1959) Can. J. Biochem. Physiol. 37, 911–920.
[23] Rinaldi-Carmona, M. et al. (1994) FEBS Lett. 350, 240–244.
[24] Dutta, A.K., Sard, H., Ryan, W., Razdan, R.K., Compton, D.R. and Martin, B.R. (1994) Med. Chem. Res. 5, 54–62.
[25] Gruol, D.L., Netzeband, J.G., Qiu, Z., Campbell, I.L. and Parsons, K.L. (1996) Adv. Exp. Med. Biol. 402, 111–117.
[26] Skaper, S.D., Buriani, A., Dal Toso, R., Petrelli, L., Romanello, S., Facci, L. and Leon, A. (1996) Proc. Natl. Acad. Sci. USA 93, 3984–3989.
[27] Caulfield, M.P. and Brown, D.A. (1992) Br. J. Pharmacol. 106, 231–232.
[28] Pan, X., Ikeda, S.R. and Lewis, D.L. (1996) Mol. Pharmacol. 49, 707–714.
[29] Hampson, A.J., Bornheim, L.M., Scanziani, M., Yost, C.S., Gray, A.T., Hansen, B.M., Leonoudakis, D.J. and Bickler, P.E. (1998) J. Neurochem. 70, 671–676.
[30] Compton, D., Rice, K., Costa, B.D., Razdan, R., Melvin, L., Johnson, M. and Martin, B. (1993) J. Pharmacol. Exp. Ther. 265, 218–226.
[31] Kearn, C.S., Greenberg, M.J., DiCamelli, R., Kurzawa, K. and Hillard, C.J. (1999) J. Neurochem. 72, 2379–2387.
[32] MacLennan, S., Reynen, P., Kwan, J. and Bonhaus, D. (1998) Br. J. Pharmacol. 124, 619–622.
[33] Leff, P. (1995) Trends Pharmacol. Sci. 16, 89–97.
[34] Lefkowitz, R.J., Cotecchia, S., Samama, P. and Costa, T. (1993) Trends Pharmacol. Sci. 14, 303–307.
[35] Levenes, C., Daniel, H., Soubrie, P. and Crepel, F. (1998) J. Physiol. 510.3, 867–879.
[36] Stefano, G.B., Liu, Y. and Goligorsky, M.S. (1996) J. Biol. Chem. 271, 19238–19242.
[37] Stefano, G.B., Salzet, B. and Salzet, M. (1997) Brain Res. 753, 219–224.
[38] Stefano, G.B., Salzet, M., Magazine, H.I. and Bilfinger, T.V. (1998) J. Cardiovasc. Pharmacol. 31, 813–820.
[39] Fimiani, C., Mattocks, D., Cavani, F., Salzet, M., Deutsch, D.G., Pryor, S., Bilfinger, T.V. and Stefano, G.B. (1999) Cell Signal 11, 189–193.