ELSEVIER

Neuroscience Letters 186 (1995) 91–94

NEUROSCIENCE LETTERS

Activation of inwardly rectifying potassium channels (GIRK1) by co-expressed rat brain cannabinoid receptors in *Xenopus* oocytes

Douglas J. Henry[a], Charles Chavkin[a,b,*]

[a]*Department of Pharmacology, SJ-30, University of Washington, Seattle, WA 98195, USA*
[b]*Division of Engineering and Applied Science, California Institute of Technology, Pasadena, CA, USA*

Received 20 December 1994; accepted 30 December 1994

Abstract

The neuronal cannabinoid receptor clone was expressed in *Xenopus laevis* oocytes as shown by the expression of saturable [^{3}H]WIN 55,212-2 binding sites. Co-expression of the cannabinoid receptor with cRNA coding for the G-protein-gated inwardly rectifying K^+ channel (GIRK1) resulted in oocytes exhibiting large inward K^+ currents in response to the cannabinoid agonist WIN 55,212-2. The activation of the potassium current by WIN 55,212-2 was dose-dependent with an EC_{50} of 630 nM. These results suggest that activation of inwardly rectifying K^+ channels may be an additional effector mechanism for brain cannabinoid receptors.

Keywords: K^+ channel; Marijuana; Addiction; Cannabis; G-Protein coupled receptor; KGA

Aside from its widespread recreational use, cannabis and its extracts have been employed therapeutically in relieving nausea, pain, and inflammation as well as the elevated intraocular pressure associated with glaucoma [8]. Cannabinoid binding sites have been demonstrated in mammalian brain [7], and receptors are most prominent in the basal ganglia, hippocampus and the cerebral and cerebellar cortices [10]. Stimulation of the neuronal cannabinoid receptor inhibits adenylate cyclase in cultured neuroblastoma cells [11], cerebellar granule neurons [19], and brain slices [1]. Since the recent cloning of neuronal [15] and peripheral [16] forms of this G-protein-coupled receptor, the intracellular signalling mechanisms of these receptors are being revealed. The neuronal cannabinoid receptor inhibits Ca^{2+} channels in a pertussis toxin (PTX)-sensitive manner in NG108-15 cells [2,14], but this action is not correlated with an alteration of intracellular cAMP levels [14]. Recently, Deadwyler et al. [6] reported a cannabinoid receptor-mediated enhancement of A-type K^+ channels in cultured hippocampal neurons. The present report describes neuronal cannabinoid receptor activation of GIRK1 (or KGA), a recently cloned G-protein-coupled inwardly rectifying K^+ channel [5,12].

The neuronal cannabinoid receptor (generously provided by Dr. Tom Bonner, NIMH) in the plasmid SKR6-14p2 [15] was amplified by PCR using synthetic oligomers designed to introduce a T7 promoter and AMV spacer sequence at the 5′ end and 30-mer poly A tail on the 3′ end of the coding sequence, as previously described [5]. The resulting cDNA template was used to prepare capped cRNA using the Megascript in vitro transcription kit protocol (Ambion Inc). The same PCR procedure was also used to produce a cDNA template of the neuronal cannabinoid receptor from a rat brain library (generous gift of Dr. Lisa DiMagno, Caltech). cRNA for GIRK1 was prepared as described [5]. Purity and yield of the products were verified by gel electrophoresis and measurement of absorbance spectra. Stage V and VI oocytes, prepared as previously described [13], were maintained at 18°C in ND96 media (mM: 96 NaCl; 2 KCl; 1 $CaCl_2$; 1 $MgCl_2$; 5 HEPES, pH 7.5) supplemented with 5% heat-inactivated horse serum, 2.5 mM sodium pyruvate and 50 μg/ml gentamycin (Sigma Chemical Co.). One day after harvest, 5 or 10 ng of cannabinoid cRNA was typically co-injected with 2 ng of GIRK1 cRNA (50 nl volume), and oocytes were tested 5–9 days later.

For radioligand binding assays, membranes were prepared as previously described [9]. Briefly, 30–60 oocytes were homogenized in 2 ml of sucrose buffer (320 mM

* Corresponding author, Fax: +1 206 685 3822; E-mail: cchavkin@u.washington.edu.

0304-3940/95/$09.50 © 1995 Elsevier Science Ireland Ltd. All rights reserved
SSDI 0304-3940(95)11289-6

sucrose, 1.0 mM EDTA, 10 mM sodium phosphate, 3 mM $MgCl_2$, pH 7.4) with protease inhibitors (0.5 μg/ml aprotinin, 10 μg/ml leupeptin and 10 μg/ml pepstatin A, Sigma Chemical Co.) and centrifuged at low speed (1000 × g, 10 min, 4°C) to remove high density debris. The supernatant was centrifuged at 100 000 × g for 30 min at 4°C. The resulting pellet was resuspended 50 mM Tris–HCl Buffer (pH 7.5) with 1 mM EDTA, 3 mM $MgCl_2$, 1 mg/ml bovine serum albumin (BSA) and protease inhibitors. Membranes (15–20 μg protein/tube, the average yield from two oocytes) were incubated with 1 nM [^{3}H]WIN 55,212-2 (Amersham) for 90 min at 22–24°C. Non-specific binding was defined by 10 μM WIN 55,212-2 (Research Biochemicals). Assays were terminated by vacuum filtration through Whatman GF/B paper saturated with 0.1% polyethylenimine and 1 mg/ml BSA, and radioactivity was quantified by liquid scintillation counting. Protein content was determined using a Bio-Rad assay with BSA as the standard.

An Axoclamp 2A amplifier was used for standard two-electrode voltage clamp experiments, in which oocytes were clamped at −80 mV. The pCLAMP program (Axon Instruments) was used for data acquisition and analysis. Because the conductance of the inward rectifier K^+ channel is dependent on both voltage and the ionic composition of the media, recordings of ligand-gated activity were made in high K^+ (hK) buffer (mM: 2 NaCl; 96 KCl; 1 $CaCl_2$; 1 $MgCl_2$; 5 HEPES, pH 7.5) [5]. BSA (3 μM) was added to all WIN 55,212-2 solutions to minimize adsorption of this compound to the perfusion system [14]. Concentrations of WIN 55,212-2 in the recording bath were verified by determining the percentage of cannabinoid adhering to the drug delivery system following perfusion of different concentrations of [^{3}H]WIN 55,212-2. EC_{50} values were calculated by log-logit transformation and linear regression of dose-response curves. For PTX experiments, oocytes were incubated for 20–24 h at 18°C in serum-free media with or without PTX (500 ng/ml) (Sigma Chemical Co.).

Oocytes expressed neuronal cannabinoid (nCANN) binding sites 5–9 days following a 5 ng injection of cRNA, exhibiting an average of 46 fmol sites/oocyte (5.8 pmol/mg protein) when incubated with 1 nM [^{3}H]WIN 55,212-2. Two assays were conducted in triplicate with oocyte batches from different frogs. Uninjected oocytes or those injected with only GIRK1 cRNA (2 ng) displayed no significant [^{3}H]WIN 55,212-2 binding. Oocytes co-injected with nCANN plus GIRK1 cRNA exhibited large (maximum 1 μA at −80 mV) inwardly rectifying K^+ currents in response to WIN 55,212-2 (Fig. 1A). Oocytes injected with nCANN cRNA prepared from the plasmid SKR6-14p2 and the rat brain library gave qualitatively similar results, and the results were pooled. This response to WIN 55,212-2 was not observed in oocytes injected with cRNA coding for the GIRK1 channel alone (2 ng, $n = 9$, Fig. 1B) or nCANN receptor alone (10 ng, $n = 3$, Fig. 1C). Oocytes injected with 10 ng nCANN receptor cRNA alone exhibited no response to 10 μM WIN 55,212-2 when a protocol that detects agonist modulation of the endogenous Ca^{2+}-activated Cl^- current [3] was utilized ($n = 4$). The current–voltage relationship for the inwardly rectifying response (Fig. 2) gave a reversal potential of −14 mV, which is similar to that observed for *kappa* opioid receptor-stimulated GIRK1 currents in

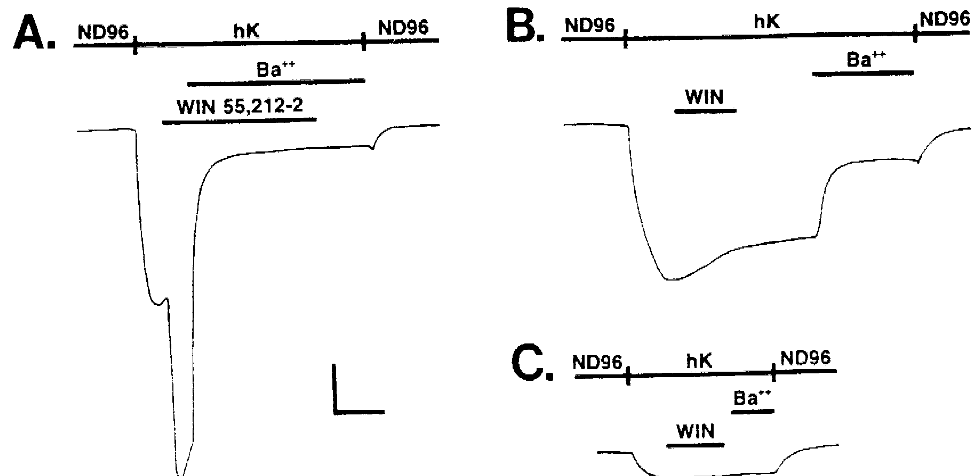

Fig. 1. (A) Inward K^+ currents evoked in oocytes injected with 10 ng nCANN plus 2 ng GIRK1 cRNA. An inwardly rectifying K^+ current was induced by exchange of ND96 buffer for high K^+ (hK) buffer in a representative oocyte voltage clamped at −80 mV. This current was enhanced by introduction of the cannabinoid agonist, WIN 55,212-2 (10 μM). Introduction of 300 μM Ba^{2+} blocked the agonist response and left a small, non-GIRK1 mediated component of the inward current response to hK. Ba^{2+} and hK were washed out simultaneously, resulting in a small inward current tail. (B) Application of the same compounds to an oocyte injected with only GIRK1 channel cRNA (2 ng) resulted in a response to hK but not WIN 55,212-2. The response to hK partially desensitized, which was observed in GIRK1-expressing oocytes in the absence of agonist. (C) An oocyte injected with only nCANN cRNA (10 ng) exhibited no response to WIN 55,212-2 and only a small, Ba^{2+}-insensitive, non-GIRK1-mediated response to hK. Vertical and horizontal scale bars represent 250 nA and 4 min, respectively, in (A) and 100 nA and 4 min in (B) and (C).

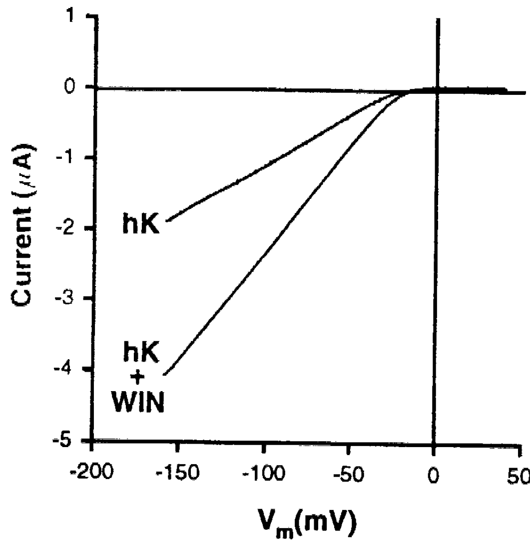

Fig. 2. The cannabinoid receptor activates the inwardly rectifying GIRK1 channel. Current–voltage relationships for the responses observed from the experiment in Fig. 1A. This plot was generated by applying a continuous voltage ramp (−160 to +40 mV over 500 ms) during peak responses to high K^+ (hK) buffer and 10 μM WIN 55,212-2 in hK. The reversal potentials for hK and hK + WIN currents were identical (−14 mV). Currents persisting in the presence of 300 μM Ba^{2+} have been subtracted from both plots.

Xenopus oocytes [9]. The WIN 55,212-2-activated response was blocked by application of 300 μM Ba^{2+} (>95% blockade, $n = 3$), a concentration shown to produce selective GIRK1 channel block [4].

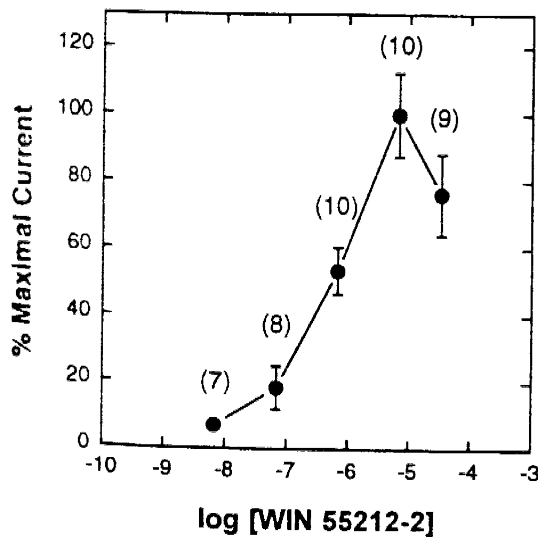

Fig. 3. Dose-response relationship for the cannabinoid agonist WIN 55,212-2 in oocytes injected with 5 ng nCANN and 2 ng GIRK1 cRNA. All oocytes were voltage clamped at −80 mV, and each was tested once with a given dose of drug. The results are normalized from two independent experiments using oocytes from different frogs. Parentheses indicate the number of oocytes tested at each dose of WIN 55,212-2. Each point represents the average peak current (±SEM) in response to hK plus agonist, minus the basal hK response, as illustrated in Fig. 1.

The EC_{50} for WIN 55,212-2 in nCANN + GIRK1-injected oocytes (630 nM, Fig. 3) was significantly higher than that reported for inhibition of Ca^{2+} channels in NG108-15 cells [14]. This may be due in part to poor penetration of the drug through the hydrophobic vitelline membrane surrounding the oocyte. However, similar lower-potency EC_{50} values have been observed for WIN 55,212-2 enhancement of A-type K^+ currents (500–1000 nM) and stimulation of a low K_m GTPase (500 nM) in cultured hippocampal neurons [6], as well as the IC_{50} for inhibition of adenylate cyclase in rat striatum (320 nM) and cerebellum (400 nM) [18]. These results suggest that NG108-15 cells may be unique in exhibiting high-affinity G-protein coupling or differing levels of spare receptors, as previously suggested by Deadwyler et al. [6].

Activation of GIRK1 by the neuronal cannabinoid receptor was accomplished without introduction of exogenous cRNA coding for G-protein, indicating that coupling between this receptor and G-protein-gated ion channel occurs via an intracellular intermediate endogenous to the oocyte. The GIRK1 channel is stimulated by G-protein $\beta\gamma$ subunits [20,21], and this may also be the mechanism coupling cannabinoid receptors to GIRK1 in oocytes. PTX treatment, which inactivates the mammalian α-subunits of G_i and G_o, had no effect on currents stimulated by 10 μM WIN 55,212-2 in oocytes (control = 68 ± 32 nA, $n = 5$; PTX-treated = 56 ± 8, $n = 4$). Previous studies have demonstrated that PTX-sensitive and insensitive forms of G_i/G_o exist in *Xenopus* oocytes [4,17]; our results suggest that nCANN-GIRK1 coupling occurs via a PTX-insensitive mechanism. The present findings suggest an additional effector system for the cannabinoid receptor which may exist in brain; the question of whether or not GIRK1 channels and cannabinoid receptors co-exist on neurons within the central nervous system awaits further study.

Supported by individual postdoctoral fellowship grant DA 05160 (D.J.H.) and grant DA 04123 (C.C.) from the National Institute on Drug Abuse. The authors thank Dr. Henry Lester and Dr. Norman Davidson for their discussion and helpful comments. We also thank Dr. Tom Bonner for the generous gift of the neuronal cannabinoid receptor clone and Dr. Lisa DiMagno for the gift of the rat brain library cDNA.

[1] Bidaut-Russell, M. and Howlett, A.C., Cannabinoid receptor-regulated cyclic AMP accumulation in the rat striatum, J. Neurochem., 57 (1991) 1769–1773.

[2] Caulfied, M.P. and Brown, D.A., Cannabinoid receptor agonists inhibit Ca current in NG108-15 neuroblastoma cells via a pertussis toxin-sensitive mechanism, Br. J. Pharmacol., 106 (1992) 231–232.

[3] Dascal, N., Ifune, C., Hopkins, R., Snutch, T.P., Lübbert, H., Davidson, N., Simon, M.I. and Lester, H.A., Involvement of a GTP-binding protein in mediation of serotonin and acetylcholine

responses in *Xenopus* oocytes injected with rat brain messenger RNA, Mol. Brain Res., 1 (1986) 201–209.

[4] Dascal, N., Lim, N.F., Schreibmayer, W., Wang, W., Davidson, N. and Lester, H.A., Expression of an atrial G-protein-activated potassium channel in *Xenopus* oocytes, Proc. Natl. Acad. Sci. USA, 90 (1993) 6596–6600.

[5] Dascal, N., Schreibmayer, W., Lim, N.F., Wang, W., Chavkin, C., DiMagno, L., LaBarca, C., Keiffer, B.L., Gaveriaux-Ruff, C., Trollinger, D., Lester, H.A. and Davidson, N., Atrial G protein-activated K^+ channel: expression cloning and molecular properties, Proc. Natl. Acad. Sci. USA, 90 (1993) 10235–10239.

[6] Deadwyler, S.A., Hampson, R.E., Bennett, B.A., Edwards, T.A., Mu, J., Pacheco, M.A., Ward, S.J. and Childers, S.R., Cannabinoids modulate potassium current in cultured hippocampal neurons, Receptors Channels, 1 (1993) 121–134.

[7] Devane, W.A., Dysarz, F.A., Johnson, M.R., Melvin, L.S. and Howlett, A.C., Determination and characterization of a cannabinoid receptor in rat brain, Mol. Pharmacol., 34 (1988) 605–613.

[8] Dewey, W.L., Cannabinoid pharmacology, Pharmacol. Rev., 38 (1986) 151–178.

[9] Henry, D.J., Grandy, D.K., Lester, H.A., Davidson, N. and Chavkin, C., Kappa opioid receptors couple to inwardly rectifying potassium channels when coexpressed by *Xenopus* oocytes, Mol. Pharmacol., in press.

[10] Herkenham, M., Lynn, A.B., Little, M.D., Johnson, M.R., Melvin, L.S., De Costa, B.R. and Rice, K.C., Cannabinoid receptor localization in brain, Proc. Natl. Acad. Sci. USA, 87 (1990) 1932–1936.

[11] Howlett, A., Cannabinoid inhibition of adenylate cyclase: biochemistry of the response in neuroblastoma cell membranes, Mol. Pharmacol., 27 (1985) 429–436.

[12] Kubo, Y., Reuveny, E., Slesinger, P.A., Jan, Y.N. and Jan, L.Y., Primary structure and functional expression of a rat G-protein-coupled muscarinic potassium channel, Nature, 364 (1993) 802–806.

[13] Leonard, J.P. and Snutch, T.P., The expression of neurotransmitter receptors and ion channels in *Xenopus* oocytes. In J. Chad and H. Wheal (Eds.), Molecular Neurobiology: A Practical Approach, IRL Press, 1991, pp. 161–182.

[14] Mackie, K. and Hille, B., Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells, Proc. Natl. Acad. Sci. USA, 89 (1992) 3825–3829.

[15] Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C. and Bonner, T.I., Structure of a cannabinoid receptor and functional expression of the cloned cDNA, Nature, 346 (1990) 561–564.

[16] Munro, S., Thomas, K.L. and Abu-Shaar, M., Molecular characterization of a peripheral receptor for cannabinoids, Nature, 365 (1993) 61–65.

[17] Olate, J., Allende, C.C., Allende, J.E., Sekura, R.D. and Birnbaumer, L., Oocyte adenylyl cyclase contains N_i, yet the guanine nucleotide-dependent inhibition by progesterone is not sensitive to pertussis toxin, FEBS Lett., 175 (1984) 25–30.

[18] Pacheco, M.A., Childers, S.R., Arnold, R., Casiano, F. and Ward, S.J., Aminoalkylindoles: actions on specific G-protein-linked receptors, J. Pharmacol. Exp. Ther., 257 (1991) 170–183.

[19] Pacheco, M.A., Ward, S.J. and Childers, S.R., Identification of cannabinoid receptors in cultures of rat cerebellar granule cells, Brain Res., 603 (1993) 102–110.

[20] Reuveny, E., Slesinger, P.A., Inglese, J., Morales, J.M., Iñiguez Lluhi, J.A., Lefkowitz, R.J., Bourne, H.R., Jan, Y.N. and Jan, L.Y., Activation of the cloned muscarinic potassium channel by G protein $\beta\gamma$ subunits, Nature, 370 (1994) 143–146.

[21] Wickman, K.D., Iñlguez-Lluhl, J.A., Davenport, P.A., Taussig, R., Kraplvinsky, G.B., Linder, M.E., Gilman, A.G. and Clapham, D.E., Recombinant G-protein $\beta\gamma$ subunits activate the muscarinic-gated atrial potassium channel, Nature, 368 (1994) 255–257.