The Journal of Neuroscience, January 1, 2000, 20(1):51–58

Cannabinoids Decrease the K^+ M-Current in Hippocampal CA1 Neurons

Paul Schweitzer

Department of Neuropharmacology, The Scripps Research Institute, La Jolla, California 92037

Cannabinoid effects on sustained conductances that control neuronal excitability have not been investigated in brain. Here, intracellular voltage-clamp recordings were performed using the rat hippocampal slice preparation to study the postsynaptic effect of cannabinoid agonists on CA1 pyramidal neurons. Superfusion of the cannabimimetics WIN55212–2 or methanandamide onto CA1 neurons elicited an inward steady-state current that reversed near the equilibrium potential for K^+ and voltage-dependently activated from a threshold of approximately −70 mV. The cannabinoid receptor (CB1) antagonist SR141716 did not alter membrane properties but prevented this effect. Further investigation revealed that the inward current elicited by cannabinoids was caused by a decrease of the noninactivating voltage-dependent K^+ M-current (I_M). Cannabinoids had no effect in slices pretreated with the M-channel blocker linopirdine. Assessment of the I_M relaxation indicated that cannabinoids decreased I_M in a concentration-dependent manner, with a maximum inhibition of 45 ± 3% with WIN55212–2 (EC_{50} of 0.6 μM) and 41 ± 5% with methanandamide (EC_{50} of 1 μM). Cannabinoids did not affect the inwardly rectifying cationic h-current (I_h). The cannabinoid-induced I_M decrease was prevented by SR141716 but remained unaffected by the muscarinic receptor antagonist atropine. Conversely, the cholinergic agonist carbamylcholine decreased I_M in the presence of SR141716, indicating that cannabinoid and muscarinic receptor activation independently diminish I_M. It is concluded that cannabinoids may postsynaptically augment the excitability of CA1 pyramidal neurons by specifically decreasing the persistent voltage-dependent I_M.

Key words: cannabinoid; brain; slice; voltage-clamp; potassium current; excitation

Cannabinoid substances have powerful psychoactive properties and alter many physiological processes, such as cognition, behavior, and nociception (Ameri, 1999). These effects are believed to be mediated via specific high-affinity binding sites present throughout the brain (Herkenham et al., 1990). A G-protein-linked receptor expressed in brain (CB1) has been cloned (Matsuda et al., 1990), and the compound SR141716 (SR1) is a selective antagonist at this receptor (Rinaldi-Carmona et al., 1994). One of the highest CB1 receptor density is found in the hippocampus, a brain structure associated with learning and memory processes, and cannabinoids appear to impair memory via activation of these receptors (Lichtman and Martin, 1996). The discovery of specific receptors led to the isolation of two endogenous ligands, the endocannabinoids anandamide (Devane et al., 1992) and 2-arachidonylglycerol (Mechoulam et al., 1995), both found in brain (Di Marzo et al., 1994; Stella et al., 1997).

Little is known on the cellular mechanisms underlying the central effects of cannabinoids, and only a few studies have been conducted at the postsynaptic level. In cultured hippocampal neurons, cannabinoid agonists increase the transient K^+ A-current (I_A) (Deadwyler et al., 1993) and reduce currents passing through N- and P/Q type calcium channels (Twitchell et al., 1997; Shen and Thayer, 1998). Cannabinoids receptors heterologously expressed in ganglion neurons also reduce Ca^{2+} currents without altering the K^+ A- and M-currents (Pan et al., 1996). Other studies using coexpression or transfection of CB1 receptors in non-neuronal systems showed that cannabinoids may also activate an inwardly rectifying K^+ conductance (Henry and Chavkin, 1995; Mackie et al., 1995). No postsynaptic studies, however, have investigated the effect of cannabinoids on sustained (noninactivating) conductances in native brain preparations, such as the hippocampal slice.

Hippocampal neurons are under the tonic control of sustained conductances, such as I_M, I_h, and leak-currents, which are active at or near resting potential and readily regulate neuronal activity (Storm, 1990). The time- and voltage-dependent I_M is modulated by several neurotransmitters and plays a unique role in modulating cellular excitability, because it is the only K^+ current that both activates below the action potential threshold and does not inactivate (Brown and Adams, 1980; Marrion, 1997). In CA1 pyramidal neurons, I_M is decreased by muscarinic agonists and serotonin (Halliwell and Adams, 1982; Colino and Halliwell, 1987) and increased by somatostatin (Moore et al., 1988). Because I_M opposes membrane depolarization, substances that decrease this current augment neuronal excitability, whereas substances that increase I_M diminish neuronal excitability.

Although sustained conductances are modulated by numerous neurotransmitters, their sensitivity to cannabinoids has not been investigated in brain. Previous postsynaptic studies have been conducted with cultured neurons or non-neuronal cells. In the present study, I recorded from native neurons in a slice preparation and found that cannabinoids reduce the K^+ I_M via activation of CB1 receptors, thus postsynaptically augmenting neuronal excitability.

Received Aug. 18, 1999; revised Oct. 1, 1999; accepted Oct. 8, 1999.

This work was funded by National Institute on Drug Abuse (NIDA) Grant K01DA00291. I thank Samuel Madamba for technical assistance and George Siggins for support (Grant DA03665 from NIDA).

Correspondence should be addressed to Dr. Paul Schweitzer, Neuropharmacology-CVN 12, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037. E-mail: pschweitzer@scripps.edu.

Copyright © 1999 Society for Neuroscience 0270-6474/99/200051-08$15.00/0

MATERIALS AND METHODS

Slice preparation. Standard intracellular recording techniques were used in rat hippocampal slices as described previously (Schweitzer et al., 1993). In brief, transverse hippocampal slices (taken from male Sprague Dawley rats of 100–170 gm) 350-μm-thick were cut on a slicer and incubated in gassed (95% O_2, 5% CO_2) artificial CSF (ACSF) of the following composition (in mM): NaCl 130, KCl 3.5, NaH_2PO_4 1.25, $MgSO_4$ 1.5, $CaCl_2$ 2.0, $NaHCO_3$ 24, and glucose 10. Slices were completely submerged and continuously superfused with warm (30–31°C) ACSF at a constant rate within the range of 1–3 ml/min. Methods of superfusion, voltage-clamp recording, drug administration, and data analysis were as described previously (Schweitzer et al., 1993). Drugs were added to the ACSF with dimethylsulfoxide (0.05–0.15% final concentration). Dimethylsulfoxide did not affect membrane properties at this concentration (Schweitzer et al., 1993). R1-methanandamide, WIN55212–2, and linopirdine (DuP 996) were purchased from Research Biochemicals (Natick, MA), tetrodotoxin was from Calbiochem (La Jolla, CA), and all other chemicals were from Sigma (St. Louis, MO). SR141716 was obtained from the National Institute of Mental Health Chemical Synthesis and Drug Supply Program.

Voltage-clamp recordings. Voltage-clamp studies were performed with an Axoclamp 2A preamplifier (Axon Instruments, Foster City, CA), using sharp glass micropipettes filled with 3 M KCl (impedance range of 50–85 MΩ) to penetrate CA1 pyramidal neurons. Tetrodotoxin (1 μM) was added to the bath after impalement to block Na^+-dependent action potentials and synaptic transmission. In discontinuous single-electrode voltage-clamp mode, the switching frequency between current injection and voltage sampling was 3–4 kHz. Current and voltage records were filtered at 0.3 kHz, acquired by analog-to-digital sampling and acquisition software, and measured with analysis software (Axon Instruments). Values are presented as mean ± SEM. The various problems associated with voltage-clamping of neurons with extended processes were discussed previously (Halliwell and Adams, 1982; Johnston and Brown, 1983). Such problems should be minimized when studying relative conductance changes with superfusion of drugs to equilibrium conditions.

Voltage protocols. Current-voltage (*I–V*) curves were generated by holding neurons at −59 ± 0.2 mV ($n = 47$) and applying hyperpolarizing and depolarizing voltage steps (1.5 sec duration, 7 sec apart). Neurons were not depolarized beyond −40 mV because of space-clamp considerations and the likelihood of activating large Ca^{2+} currents. *I–V* curves were constructed from current values measured at the end of the voltage step (steady state), and the values obtained in control condition were subtracted from those in presence of the tested substances to obtain the net current induced. Two voltage-dependent noninactivating conductances found in CA1 neurons were separately assessed. The I_M relaxation was observed at the onset of hyperpolarizing voltage steps (1 sec duration) delivered from a holding potential (V_H) of −44 ± 0.3 mV ($n = 49$). The I_h relaxation was observed at the onset of hyperpolarizing voltage steps delivered from a holding potential of −59 mV (Halliwell and Adams, 1982).

RESULTS

Intracellular recordings were performed from 65 CA1 pyramidal neurons using the adult hippocampal slice preparation to investigate cannabinoid effects on sustained conductances. The average resting membrane potential (RMP) was −69 ± 0.3 mV, the input resistance determined at onset of a small hyperpolarizing current step before addition of tetrodotoxin was 74 ± 2 MΩ, and the action potential amplitude from threshold was 104 ± 1 mV. Two nondegradable cannabinoid agonists were used: the methylated analog of anandamide R1-methanandamide (mAEA), and the aminoalkyndole WIN55212–2 (WIN-2).

Cannabinoids elicit an inward steady-state current

I–V relationships were generated to study the effects of cannabinoids on steady-state membrane properties in the depolarized and hyperpolarized ranges. Superfusion of mAEA (5 μM) onto CA1 pyramidal neurons elicited an inward steady-state current in the depolarized range but showed no effect at hyperpolarized potentials (Fig. 1*A*). Current values were back near control upon washout of the drug. The net steady-state currents were obtained

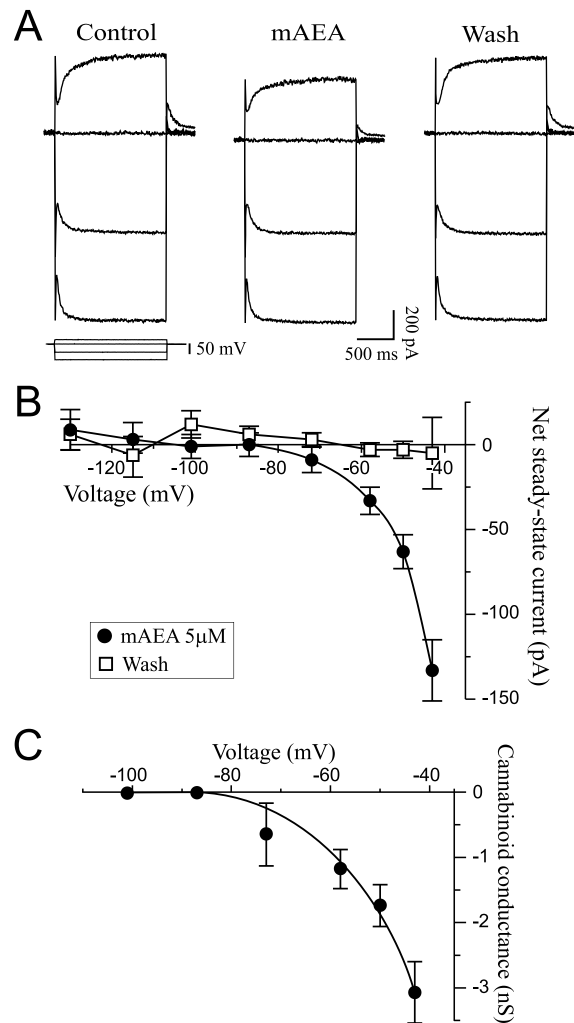

Figure 1. Cannabinoids elicit an inward steady-state current. *A*, Selected current traces obtained with an *I–V* protocol. This representative CA1 pyramidal neuron held at −56 mV was subjected to three different voltage steps sequentially applied and superimposed at each condition (voltage protocol at *bottom left*). Superfusion of 5 μM mAEA induced an inward steady-state current at depolarized potentials (170 pA at −42 mV) but had no effect in the hyperpolarized range. RMP was −69 mV. *B*, Net currents averaged from five neurons exposed to 5 μM mAEA. The cannabinoid elicited a voltage-dependent inward current that reversed at −87 mV, with recovery to control values on washout of the drug. *C*, Plot of the mAEA-induced conductance derived from *B*. G_{mAEA} was calculated as $I_{mAEA}/(V - V_{rev})$, where I_{mAEA} is the mAEA-induced current, V is the command potential, and V_{rev} is the reversal potential. The conductance was voltage-dependent and activated at approximately −75 mV.

by subtracting current values obtained at each condition from current values in control (Fig. 1*B*). The mAEA component showed voltage-dependence and had a reversal potential of −87 ± 5 mV ($n = 5$), close to the theoretical equilibrium potential for K^+ (−98 mV in these experimental conditions). The conductance decrease elicited by mAEA, G_{mAEA}, was calculated by dividing the cannabinoid-induced current by the driving force (Fig. 1*C*). G_{mAEA} was voltage-dependent with an activation threshold of approximately −75 mV and amplitude of −3.1 nS at −43 mV. The mAEA effect was dose-dependent as the amplitude of the inward current increased with the drug concentration (Fig. 2). The apparent threshold response was 0.25 μM, and the maximum effect was obtained with 5 μM mAEA.

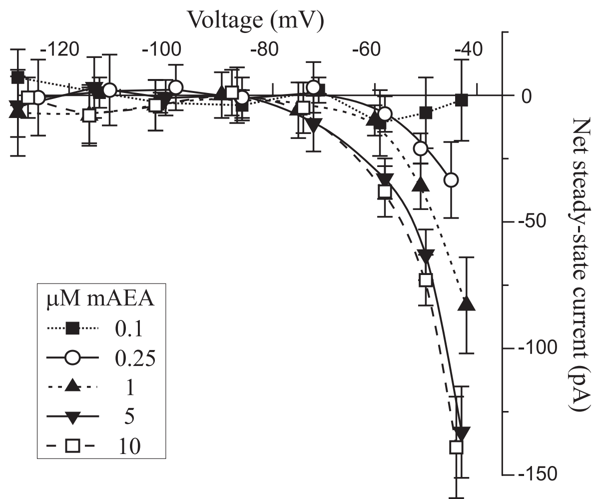

Figure 2. The cannabinoid inward current is concentration-dependent. Averaged steady-state currents elicited with different concentrations of mAEA: 0.1 μM (n = 3), 0.25 μM (n = 4), 1 μM (n = 4), 5 μM (n = 5), and 10 μM (n = 4). The amplitude of the inward current increased with the concentration of mAEA. The threshold response was 0.25 μM, and the maximum effect was reached with 5 μM.

It was then determined whether the mAEA effect was mediated via activation of CB1 receptors by using the selective CB1 receptor antagonist SR1. Superfusion of SR1 alone (1 μM) did not elicit a measurable effect on steady-state currents throughout the potential range tested (Fig. 3*A*,*B*). However, the mAEA-induced component was completely prevented by SR1, indicating that the cannabinoid effect occurred via activation of CB1 receptors. To confirm these findings, the experiments were repeated with the structurally different cannabinoid WIN-2. WIN-2 had effects similar to those of mAEA and induced a voltage-dependent inward current that reversed at −85 mV (Fig. 3*C*). The threshold response was 0.25 μM and the maximum inward current was obtained with 2 μM (n = 6), because superfusion of 5 μM WIN-2 did not elicit a larger effect (n = 3; data not shown). The maximum effect, however, was not as pronounced and consistent as the effect observed with mAEA, although it occurred at a lesser concentration. The effect of WIN-2 was also prevented by SR1 (Fig. 3*C*), demonstrating involvement of CB1 receptors.

Cannabinoids decrease I_M

The I_M is a persistent voltage-dependent K^+ outward current that activates at approximately −70 mV, thus having properties resembling the effect elicited by cannabinoids. A separate voltage protocol was used to quantify I_M (see Materials and Methods) and determine whether cannabinoids decreased I_M to elicit the observed inward steady-state current at depolarized potentials. Addition of WIN-2 in the superfusate indeed reduced I_M relaxation amplitudes (Fig. 4*A*) and concomitantly elicited an inward holding current (Fig. 4*A*, *dotted line*), consistent with closing of M-channels. All values returned toward control levels upon washout of WIN-2, although recovery was only partial. The averaged effect on I_M over nine neurons is shown on Figure 4*B*; WIN-2 (2–5 μM) decreased I_M to 55 ± 3% of control, with a recovery on washout to 85 ± 6% of control. The specific I_M blocker linopirdine (Aiken et al., 1995) was used to further identify I_M as the target of the cannabinoid effect. Linopirdine elicited an inward steady-state current because of I_M inhibition (Fig. 4*C*) and decreased I_M relaxations to 18 ± 4% of control (n = 5; data not shown). Addition of 2 μM WIN-2 in the continued presence of linopirdine did not alter steady-state currents (Fig. 4*C*) or I_M relaxations that remained at 17 ± 4% of control, indicating that cannabinoids solely affected I_M.

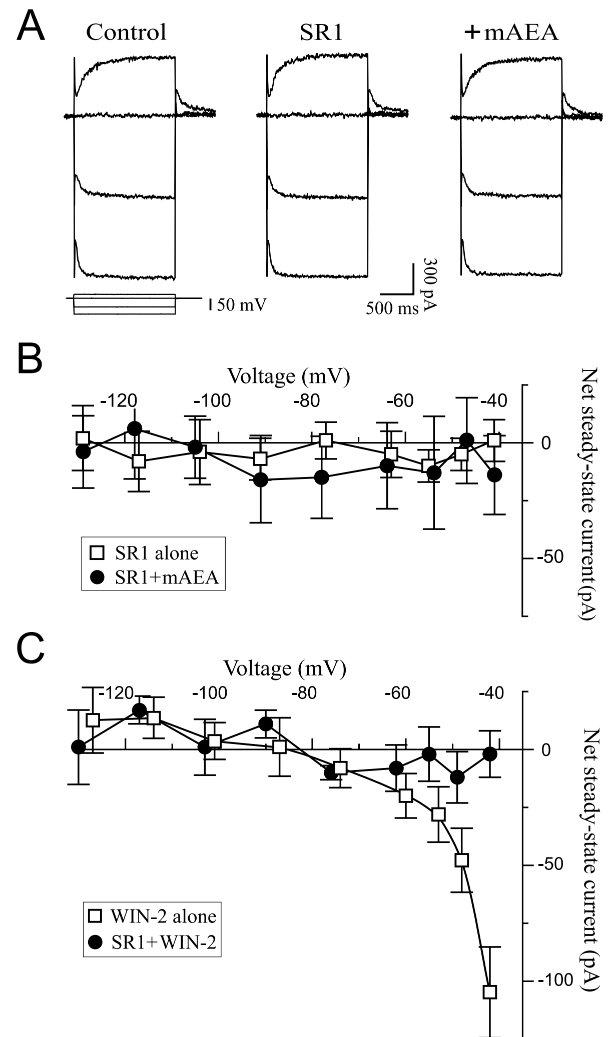

Figure 3. The cannabinoid inward current is elicited via activation of CB1 receptors. *A*, Selected current traces from a neuron exposed to the CB1 antagonist SR1 (1 μM) and mAEA (5 μM) in the presence of SR1. SR1 alone had no effect but completely prevented the mAEA response. RMP was −67 mV, and V_H was −59 mV. *B*, Net currents averaged from seven neurons exposed to 1 μM SR1 alone and three neurons exposed to 5–10 μM mAEA in the presence of SR1. The antagonist completely prevented the mAEA effect. *C*, Net currents elicited by WIN-2 in the absence (2 μM; n = 6) or presence (2–5 μM; n = 5) of 1 μM SR1. WIN-2 elicited a voltage-dependent inward current that was completely prevented by SR1.

Cannabinoids reportedly augment inwardly rectifying K^+ conductances in expression systems. I investigated a possible action of cannabinoids on I_h (also called I_Q), a persistent Na^+–K^+ conductance that activates in the hyperpolarized range below −60 mV and rectifies inwardly. The I_h relaxation amplitude was unchanged upon exposure to mAEA (Fig. 4*D*) or WIN-2 (data not shown). On average, I_h remained at 99 ± 3% of control when neurons were exposed to 5–10 μM mAEA (n = 6) and 101 ± 2% of control when 2–5 μM WIN-2 was applied (n = 8).

The cannabinoid-induced I_M decrease was concentration-dependent. Superfusion of 1 μM mAEA decreased the I_M ampli-

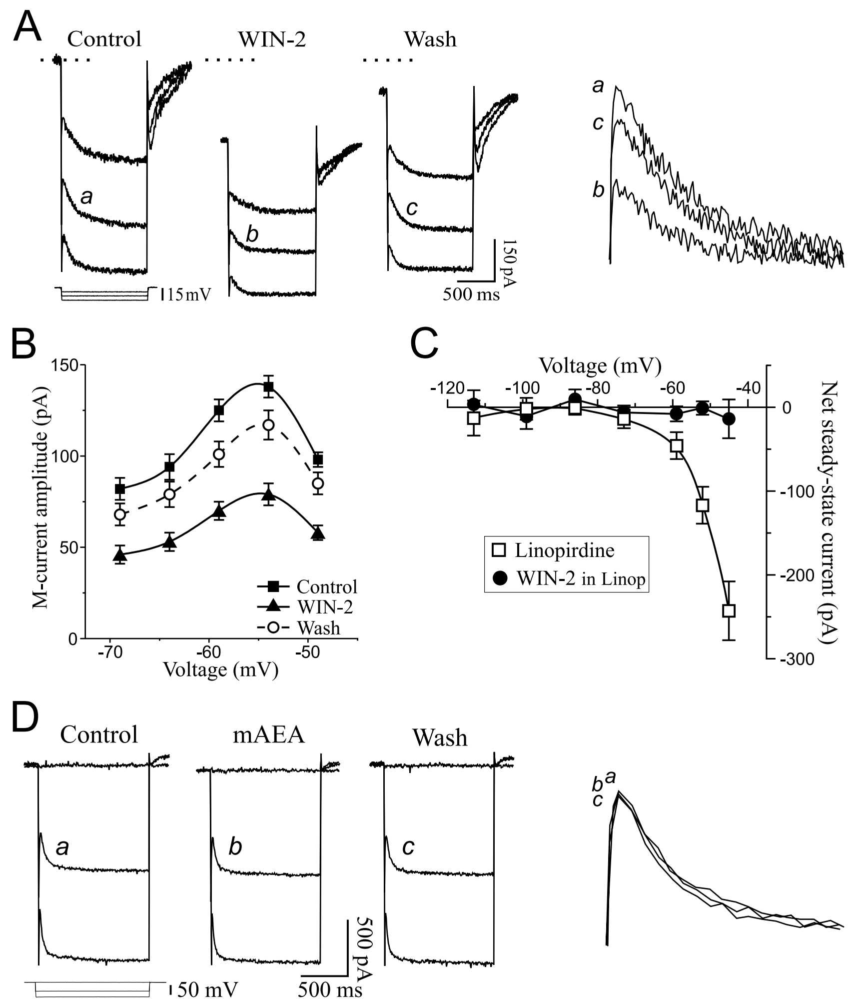

Figure 4. Cannabinoids decrease I_M. *A*, Current recordings showing I_M relaxations from a neuron held at −44 mV. Hyperpolarizing voltage commands (3 steps superimposed, protocol at *bottom left*) were applied to deactivate I_M (slow relaxation at command onset). WIN-2 elicited an I_M decrease associated with an inward holding current (*dotted line* is control holding current). The I_M relaxations identified with *letters* are magnified and superimposed on the *far right* for comparison. RMP was −67 mV. *B*, Average of I_M amplitude in nine cells tested with 2–5 μM WIN-2. The cannabinoid decreased I_M by 44% with recovery to 85% of control upon washout. *C*, Net steady-state currents from five neurons exposed to the selective I_M inhibitor linopirdine, followed by WIN-2. Linopirdine (10 μM) elicited a voltage-dependent inward current because of blockade of M-channels. Further addition of 2 μM WIN-2 had no effect, indicating that cannabinoids affected only I_M. *D*, Recordings showing I_h relaxations observed with hyperpolarizing voltage commands to −103 and −119 mV (V_H of −58 mV). Superfusion of 5 μM mAEA did not alter I_h amplitude. RMP was −68 mV.

tude by 27% and elicited a small inward holding current (Fig. 5*A*). A higher concentration of 5 μM mAEA elicited a stronger effect to decrease I_M by 58%, concomitant with a large inward holding current (Fig. 5*B*). Current values returned near control levels on washout of mAEA. The dose–response relationship obtained with WIN-2 and mAEA is shown in Figure 5*C*. WIN-2 had a maximal effect at 3 μM to decrease I_M to 55% of control, with an apparent EC_{50} of 0.6 μM. The maximal effect with mAEA was obtained at 6 μM to decrease I_M to 59% of control, with an apparent EC_{50} of 1 μM.

Cannabinoids decrease I_M via CB1 receptors independently of muscarinic receptors

The CB1 receptor antagonist SR1 was used to determine whether the cannabinoid-induced I_M decrease occurred via activation of CB1 receptors. Superfusion of 1 μM SR1 alone had no effect on I_M amplitude ($n = 5$; data not shown). In the presence of SR1, however, a subsequent application of WIN-2 at concentrations that greatly reduced I_M (1–5 μM; $n = 5$) was without effect (Fig. 6*A*,*B*). Likewise, mAEA (5–10 μM; $n = 3$) did not affect I_M nor elicit an inward holding current in slices pretreated with SR1 (Fig. 6*C*), indicating that cannabinoids decreased I_M by activating CB1 receptors.

A possible involvement of muscarinic receptors in the cannabinoid effect was investigated by treating the slices with the muscarinic receptor antagonist atropine. In the presence of 1 μM atropine, the nondegradable cholinergic agonist carbamylcholine (carbachol, 5 μM) did not affect I_M because of blockade of muscarinic receptors. Addition of 2 μM WIN-2 in the presence of atropine, however, greatly decreased the I_M relaxation (Fig. 7*A*). On average, atropine alone did not affect I_M, but addition of WIN-2 together with atropine decreased I_M to 56 ± 5% of control ($n = 5$) (Fig. 7*B*), a value similar to that observed in the absence of the muscarinic antagonist (55 ± 3% of control) (Fig. 4*B*). To ensure that the well known muscarinic-induced I_M inhibition occurred independently of CB1 receptors, additional experiments were conducted with SR1 and carbachol. In the presence of the cannabinoid receptor antagonist, WIN-2 no longer altered I_M, but further addition of 5 μM carbachol greatly decreased I_M (Fig. 7*C*). On average, carbachol was more efficacious than cannabinoids and decreased I_M to 20 ± 6% of control ($n = 4$; 15 mV hyperpolarizing step). These results show that cannabinoid and muscarinic receptor agonists independently diminish I_M.

The cannabinoid effects on the I_M relaxation are summarized for comparison in Figure 8. WIN-2 decreased I_M by 45 ± 3% when applied alone and by 44 ± 5% in the presence of atropine. SR1 alone did not affect I_M (2 ± 3% increase) but prevented WIN-2 from inhibiting I_M (3 ± 5% decrease). Similar to WIN-2, mAEA decreased I_M by 41 ± 5% in absence of SR1 and by 6 ± 6% when the CB1 receptor antagonist was present.

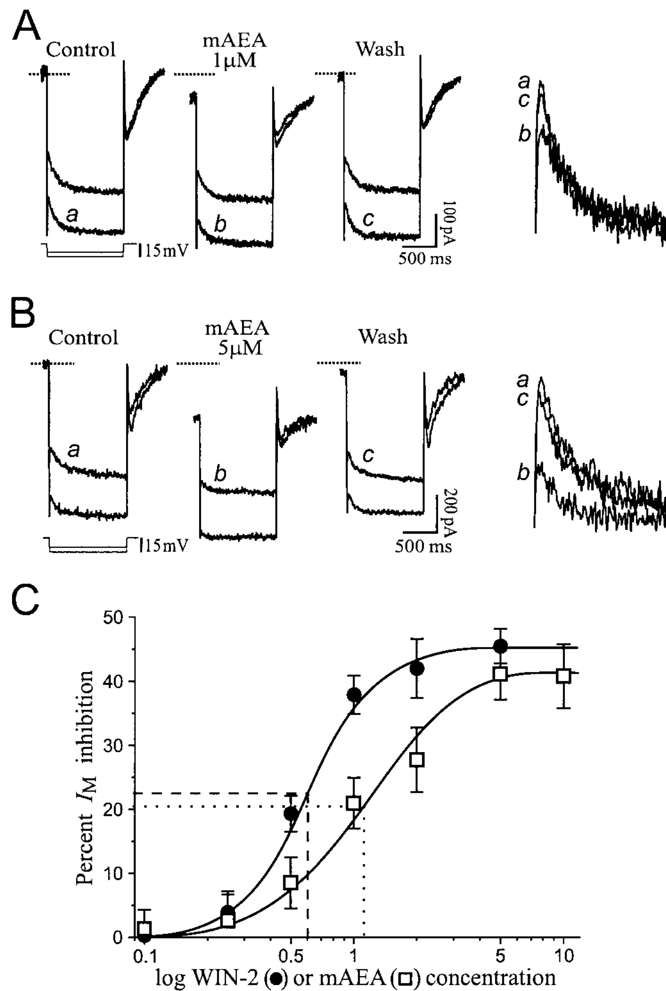

Figure 5. The cannabinoid-induced I_M decrease is concentration-dependent. *A*, I_M recordings from a neuron exposed to 1 μM mAEA. Superfusion of mAEA decreased I_M by 27% (I_M relaxations magnified on the *far right*) and elicited a limited inward holding current. RMP was −68 mV, and V_H was −47 mV. *B*, Superfusion of 5 μM mAEA produced a larger I_M decrease (by 58% on this cell; relaxations magnified on *far right*) associated with a pronounced inward holding current. RMP was −71 mV, and V_H was −43 mV. *C*, Dose–response curve of I_M inhibition by WIN-2 (*filled circles*) or mAEA (*open squares*). The threshold response was below 0.2 μM, and maximal effects were obtained with 3 μM WIN-2 (EC_{50} of 0.6 μM; *dashed line*) to inhibit I_M by 45% and 6 μM mAEA (EC_{50} of 1 μM; *dotted line*) to inhibit I_M by 41%.

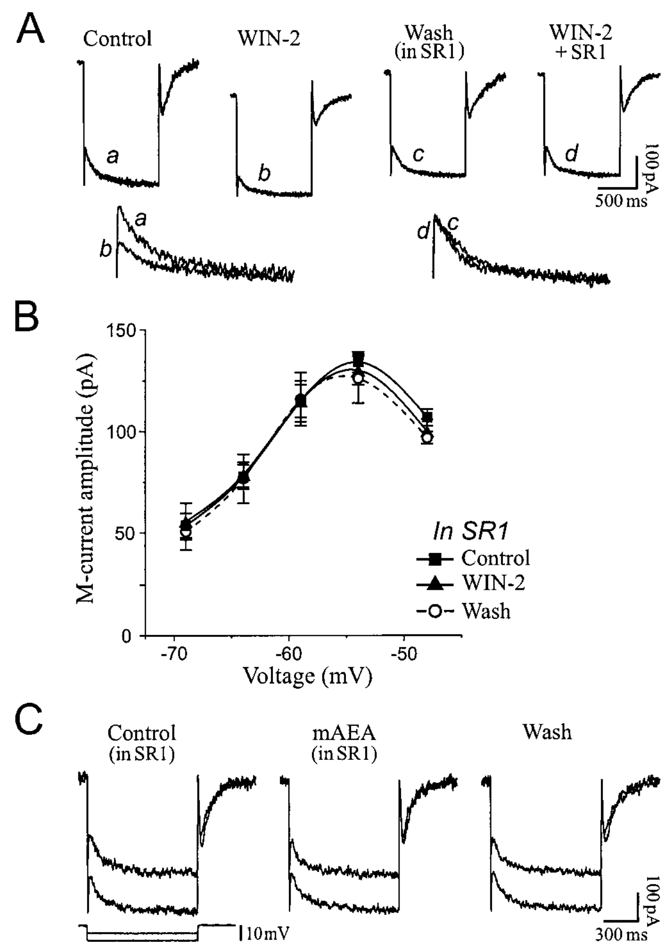

Figure 6. The cannabinoid-induced I_M decrease is mediated via CB1 receptors. *A*, I_M relaxation elicited with a 10 mV hyperpolarizing step (V_H of −42 mV). A first application of 1 μM WIN-2 decreased I_M by 47%. After washout of WIN-2 coincident with superfusion of 1 μM SR1, a second application of WIN-2 in the continued presence of SR1 had no effect on I_M. The *bottom panel* shows the magnified I_M relaxations. RMP was −71 mV. *B*, Average of I_M amplitude on five neurons exposed to 1–5 μM WIN-2 in slices treated with 1 μM SR1, showing the lack of effect of the cannabinoid in presence of the CB1 antagonist. *C*, SR1 also prevented the I_M decrease expected with superfusion of 5 μM mAEA. RMP was −67 mV, and V_H was −48 mV.

DISCUSSION

The results showed that cannabinoids acting at CB1 receptors elicited a postsynaptic excitatory effect on CA1 pyramidal neurons by decreasing the persistent voltage-dependent I_M.

Cannabinoids decrease the persistent I_M

In the presence of tetrodotoxin to block neurotransmission, cannabinoids elicited an inward current that voltage-dependently increased with depolarization. The current reversed at −87 mV, indicating that K^+ was the carrier, and activated at approximately −75 mV. Such properties were reminiscent of I_M, a time- and voltage-dependent persistent K^+ current that activates between −80 and −70 mV, and the *I–V* relationship profile of the cannabinoid effect was consistent with a decrease of I_M. Although the inwardly rectifying I_h activates only at hyperpolarized potentials, the I_M and I_h relaxations appear similar. The results showed that neither WIN-2 nor mAEA altered I_h. Moreover, WIN-2 had no effect on neurons pretreated with the M-channel blocker linopirdine (Aiken et al., 1995), verifying that cannabinoids solely affected I_M. However, *I–V* relationships were not performed beyond −40 mV because of space-clamp considerations, and a cannabinoid action on conductances active at more depolarized potentials is possible.

The cannabinoid effect was dose-dependent. WIN-2 and mAEA had a comparable efficacy and decreased I_M to a similar level, although WIN-2 appeared more potent. The EC_{50} values of 0.6 and 1 μM are comparable with the 1–2 μM range reported for synaptic inhibition in brain slices (Lévénès et al., 1998; Szabo et al., 1998) but much higher than the 10–20 nM range reported for Ca^{2+} current inhibition in hippocampal cultures (Twitchell et al., 1997; Shen and Thayer, 1998). Such discrepancy is usually attributed to limited drug penetration and inferior access to the recorded neurons in slice preparations.

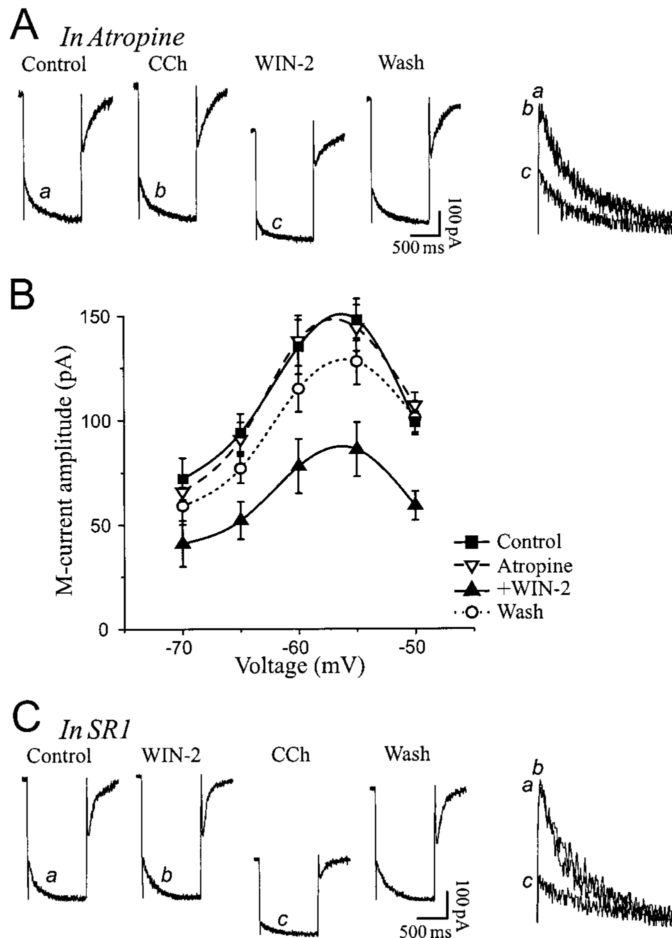

Figure 7. Cannabinoid and muscarinic receptor agonists independently decrease I_M. *A*, I_M relaxation elicited with a 10 mV hyperpolarizing step (V_H of −47 mV) in the presence of the muscarinic receptor antagonist atropine (1 μM). Carbachol (*CCh*, 5 μM) had no effect on I_M because of blockade of muscarinic receptors, but addition of 2 μM WIN-2 in the continued presence of atropine decreased I_M. RMP was −67 mV. *B*, Average I_M amplitude on five cells exposed to 1 μM atropine, followed by 2 μM WIN-2. The cannabinoid-induced I_M decrease was unaffected by the muscarinic receptor antagonist. *C*, I_M relaxation elicited with a 10 mV hyperpolarizing step (V_H of −44 mV) in the presence of SR1. WIN-2 had no effect on I_M because of blockade of CB1 receptors, but 5 μM CCh decreased I_M (washout performed in atropine). RMP was −69 mV.

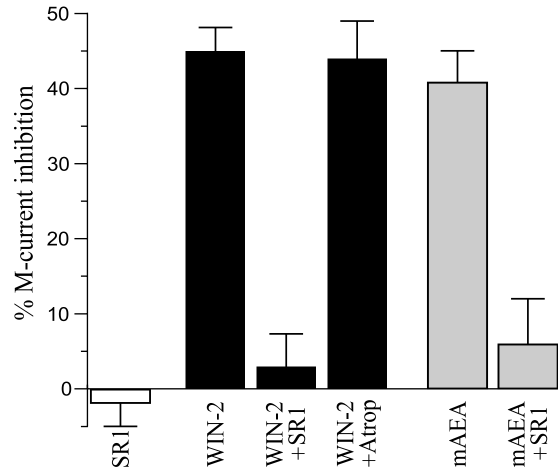

Figure 8. Summary chart of I_M inhibition by cannabinoids. Superfusion of SR1 alone did not affect I_M amplitude (2% augmentation). WIN-2 decreased I_M by 45%, an effect prevented in the presence of SR1 (3% decrease) but unaltered by atropine (44% decrease). Comparable results were obtained with mAEA that decreased I_M by 41% in absence of SR1 and by 6% in presence of the CB1 antagonist.

Cannabinoid and muscarinic receptor activation independently decrease I_M

The inward steady-state current and I_M decrease elicited by mAEA and WIN-2 were both prevented in slices treated with SR1, demonstrating that cannabinoids activated CB1 receptors. A previous report showed that endocannabinoids are detected in hippocampal slices subjected to similar experimental conditions, including the presence of tetrodotoxin (Stella et al., 1997). In the present study, SR1 applied alone had no effect on the recorded currents, indicating that endocannabinoids may not tonically affect postsynaptic properties in the slice preparation.

Cholinergic agonists acting at muscarinic receptors decrease I_M. Because cannabinoids have been shown to inhibit the release of acetylcholine in hippocampus (Gifford and Ashby, 1996) and carbachol reportedly enhances the production of the endocannabinoid 2-arachidonylglycerol in rat aorta (Mechoulam et al., 1998b), experiments using receptor antagonists were conducted to investigate possible interactions. The presence of atropine did not alter the extent of I_M inhibition by WIN-2. Conversely, carbachol decreased I_M in the presence of SR1, indicating that cannabinoid and muscarinic receptor agonists independently decrease I_M.

Postsynaptic actions of cannabinoids

The cannabinoid modulation of persistent conductances has not been investigated in brain neurons, precluding an adequate comparison with the present effect. In cultured hippocampal neurons, cannabinoids augment the transient K^+ I_A and may therefore modulate the excitatory synaptic input (Deadwyler et al., 1995). Although this conductance does not readily influence neuronal activity, its augmentation denotes an inhibitory action of cannabinoids. Experiments conducted in non-neuronal expression systems showed that cannabinoids increased an inwardly rectifying K^+ conductance (Henry and Chavkin, 1995; Mackie et al., 1995). The augmentation of such conductance generates a small outward current to inhibit neuronal activity, in contrast to the present results that point to increased excitability. Such differences can be explained by the use of totally different preparations, native brain slices versus non-neuronal systems expressing CB1 receptors. As well, the lack of effect of cannabinoids on I_M and I_A in ganglion neurons transiently expressing CB1 receptors may be because of an ineffective coupling of the adequate second messenger systems (Pan et al., 1996).

The identification of the intracellular mechanisms of I_M inhibition remains under intense investigation. A rise of intracellular Ca^{2+} levels may play a key role in the decrease of I_M by various transmitters (for review, see Marrion, 1997). Cannabinoids can increase intracellular Ca^{2+} levels via phospholipase C in cell lines (Sugiara et al., 1997). Cannabinoids also enhance the depolarization-induced increase of intracellular Ca^{2+} by a mechanism involving phospholipase C and Ca^{2+} release from inositol triphosphate-sensitive Ca^{2+} stores in cerebellar neurons (Netzeband et al., 1999). Interestingly, a recent study showed that bradykinin inhibits I_M in ganglion neurons via phospholipase C and Ca^{2+} release from inositol triphosphate-sensitive Ca^{2+} stores (Cruzblanca et al., 1998). Such a mechanism could be

involved in the cannabinoid inhibition of I_M in CA1 pyramidal neurons in which an increase in intracellular concentrations of inositol triphosphate reportedly decrease I_M (Dutar and Nicoll, 1988).

Cannabinoids and eicosanoids have opposite effects

Arachidonic acid and its metabolites, the eicosanoids, are potent signaling molecules implicated in several forms of neuromodulation (Meves, 1994; Piomelli, 1994). Although arachidonic acid is produced upon degradation of anandamide and 2-arachidonylglycerol (Mechoulam et al., 1998a), the fatty acid and its lipoxygenase metabolites augment I_M in CA1 pyramidal neurons (Schweitzer et al., 1990), an effect opposite to those of cannabinoids. Interestingly, arachidonic acid also decreases the hippocampal I_A (Keros and McBain, 1997), whereas cannabinoids increase it (Deadwyler et al., 1993). Furthermore, cannabinoids prevent hippocampal long-term potentiation (Collins et al., 1994; Stella et al., 1997), whereas arachidonic acid elicits this phenomenon (Williams et al., 1989). Thus, cannabinoids and eicosanoids act on similar targets in hippocampus but in an opposite direction.

The arachidonic acid produced upon endocannabinoid degradation has to be rapidly removed to prevent further biological effects. Indeed, very little arachidonic acid resulting from endocannabinoid hydrolysis is detected using cellular assays, because the fatty acid appears to be immediately reincorporated into membrane phospholipids (Mechoulam et al., 1998a). The I_M decrease via arachidonic acid activation of protein kinase C reported in cultured cells (Schmitt and Meves, 1993) is also an unlikely mechanism, especially because a recent study indicates that stimulation of protein kinase C phosphorylates CB1 receptors and prevents cannabinoid actions (Garcia et al., 1998). Evidently, the eicosanoids do not mediate cannabinoid effects. Still, the fact that these two closely related families of lipidic mediators have opposite effects is puzzling.

Functional implications

Because I_M is a persistent current active near the threshold for action potential initiation, it has a major influence on neuronal excitability and responsiveness to synaptic inputs (Marrion, 1997). The primary role of I_M is to clamp the membrane potential near rest. When depolarizing events occur, I_M activates to hyperpolarize the membrane back toward resting potential and prevents excessive depolarizations. Thus, I_M participates in the mechanism of spike frequency adaptation to slow the firing of action potentials (Aiken et al., 1995) and also plays a major role in the termination of bursting activity in CA1 neurons (Azouz et al., 1996). By reducing I_M, cannabinoids diminish the ability of neurons to counteract depolarizations, favoring increased firing of action potentials and prolonged bursting.

Interestingly, cannabinoids reinforce bursting activity in CA1 hippocampus (Xue et al., 1993) and increase neuronal firing rate and bursting activity in the ventral tegmentum and substantia nigra pars compacta *in vivo* (French et al., 1997), an effect consistent with I_M inhibition. An alteration of I_M could also be involved in the dual effects of cannabinoids on neurons of the solitary tract nucleus (Himmi et al., 1998). The present results indicate that, in addition to presynaptic disinhibitory effects associated with decreased GABAergic transmission (Miller and Walker, 1995; Chan and Yung, 1998; Szabo et al., 1998), cannabinoids may also directly increase neuronal activity via postsynaptic actions. It should be noted, however, that hippocampal pyramidal neurons reportedly possess few CB1 receptors (Tsou et al., 1998), and an indirect effect is always possible despite the blockade of neurotransmission by tetrodotoxin.

Recent reports have attributed the occurrence of an epileptic syndrome to mutations of the K^+ channel genes KCNQ2 and KCNQ3 (Biervert et al., 1998; Charlier et al., 1998). Further work demonstrated that the combination of KCNQ2 and KCNQ3 subunits, highly expressed in hippocampus, form native M-channels (Wang et al., 1998). These data strongly implicate I_M in the control of seizure. Cannabinoid research performed before the identification of specific receptors showed that Δ^9-tetrahydrocannabinol has both convulsant and anticonvulsant effects (for review, see Martin, 1986). Although the mechanisms implicated in these actions were not determined, the anticonvulsant effect could be possibly attributed to the cannabinoid inhibition of glutamate release (Ameri, 1999). On the other hand, and consistent with the alteration of M-channel expression in some form of epilepsy, the cannabinoid inhibition of I_M could play a role in the reported convulsant action.

Conclusion

The activation of CB1 receptors postsynaptically decreases I_M in CA1 pyramidal neurons. This action will diminish the ability of neurons to counteract depolarizing events and may play an important role in response to hyperexcitability and bursting in hippocampus. Cannabinoids can therefore increase neuronal excitability by altering I_M but can also decrease hippocampal activity by inhibiting neurotransmitter release and synaptic plasticity. Surprisingly, cannabinoids and eicosanoids have opposite effects on hippocampal electrophysiology.

REFERENCES

Aiken SP, Lampe BJ, Murphy PA, Brown BS (1995) Reduction of spike frequency adaptation and blockade of M-current in rat CA1 pyramidal neurones by linopirdine (DuP 996), a neurotransmitter release enhancer. Br J Pharmacol 115:1163–1168.

Ameri A (1999) The effects of cannabinoids on the brain. Prog Neurobiol 58:315–348.

Azouz R, Jensen MS, Yaari Y (1996) Ionic basis of spike after-depolarization and burst generation in adult rat hippocampal CA1 pyramidal cells. J Physiol (Lond) 492:211–223.

Biervert C, Schroeder BC, Kubisch C, Berkovic SF, Propping P, Jentsch TJ, Steinlein OK (1998) A potassium channel mutation in neonatal human epilepsy. Science 279:403–406.

Brown DA, Adams PR (1980) Muscarinic suppression of a novel voltage-sensitive K^+ current in a vertebrate neurone. Nature 283:673–676.

Chan PK, Yung WH (1998) Occlusion of the presynaptic action of cannabinoids in rat substantia nigra pars reticulata by cadmium. Neurosci Lett 249:57–60.

Charlier C, Singh NA, Ryan SG, Lewis TB, Reus BE, Leach RJ, Leppert M (1998) A pore mutation in a novel KQT-like K^+ channel gene in an idiopathic epilepsy family. Nat Genet 18:53–55.

Colino A, Halliwell JV (1987) Differential modulation of three separate K^+ conductances in hippocampal CA1 neurons by serotonin. Nature 328:73–77.

Collins DR, Pertwee RG, Davies SN (1994) The action of synthetic cannabinoids on the induction of long-term potentiation in the rat hippocampal slice. Eur J Pharmacol 259:R7–R8.

Cruzblanca H, Koh DS, Hille B (1998) Bradykinin inhibits M-current via phospholipase C and Ca^{2+} release from IP_3-sensitive Ca^{2+} stores in rat sympathetic neurons. Proc Natl Acad Sci USA 95:7151–7156.

Deadwyler SA, Hampson RE, Bennett BA, Edwards TA, Mu J, Pacheco MA, Ward SJ, Childers SR (1993) Cannabinoids modulate potassium current in cultured hippocampal neurons. Receptors Channels 1:121–134.

Deadwyler SA, Hampson RE, Mu J, Whyte A, Childers S (1995) Cannabinoids modulate voltage sensitive potassium A-current in hippocampal neurons via a cAMP-dependent process. J Pharmacol Exp Ther 273:734–743.

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258:1946–1949.

Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, Schwartz J-C, Piomelli D (1994) Formation and inactivation of endogenous cannabinoid anandamide in central neurons. Nature 372:686–691.

Dutar P, Nicoll RA (1988) Classification of muscarinic responses in hippocampus in terms of receptor subtypes and second-messenger systems: electrophysiological studies *in vitro*. J Neurosci 8:4214–4224.

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

Garcia DE, Brown S, Hille B, Mackie K (1998) Protein kinase C disrupts cannabinoid actions by phosphorylation of the CB1 cannabinoid receptor. J Neurosci 18:2834–2841.

Gifford AN, Ashby Jr CR (1996) Electrically evoked acetylcholine release from hippocampal slices is inhibited by the cannabinoid receptor agonist, WIN 55212–2, and is potentiated by the cannabinoid antagonist, SR 141716A. J Pharmacol Exp Ther 277:1431–1436.

Halliwell JV, Adams PR (1982) Voltage-clamp analysis of muscarinic excitation in hippocampal neurons. Brain Res 250:71–92.

Henry DJ, Chavkin C (1995) Activation of inwardly rectifying potassium channels (GIRK1) by co-expressed rat brain cannabinoid receptors in *Xenopus* oocytes. Neurosci Lett 186:91–94.

Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, De Costa BR, Rice KC (1990) Cannabinoid receptor localization in brain. Proc Natl Acad Sci USA 87:1932–1936.

Himmi T, Perrin J, El Ouazzani T, Orsini JC (1998) Neuronal responses to cannabinoid receptor ligands in the solitary tract nucleus. Eur J Pharmacol 359:49–54.

Johnston D, Brown TH (1983) Interpretation of voltage-clamp measurements in hippocampal neurons. J Neurophysiol 50:464–486.

Keros S, McBain CJ (1997) Arachidonic acid inhibits transient potassium currents and broadens action potentials during electrographic seizures in hippocampal pyramidal and inhibitory interneurons. J Neurosci 17:3476–3487.

Lévénès C, Daniel H, Soubrié P, Crépel F (1998) Cannabinoids decrease excitatory synaptic transmission and impair long-term depression in rat cerebellar Purkinje cells. J Physiol (Lond) 510:867–879.

Lichtman AH, Martin BR (1996) Delta^9-tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism. Psychopharmacology 126:125–131.

Mackie K, Lai Y, Westenbroek R, Mitchell R (1995) Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor. J Neurosci 15:6552–6561.

Marrion NV (1997) Control of M-current. Annu Rev Physiol 59:483–504.

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

Matsuda LA, Lolait J, Brownstein MJ, Young AC, Bonner TI (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346:561–564.

Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR, Pertwee RG, Griffin G, Bayewitch M, Barg J, Vogel Z (1995) Identification of an endogenous 2-mono-glyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol 50:83–90.

Mechoulam R, Fride E, Di Marzo V (1998a) Endocannabinoids. Eur J Pharmacol 359:1–18.

Mechoulam R, Fride E, Ben-Shabat S, Meiri U, Horowitz M (1998b) Carbachol, an acetylcholine receptor agonist, enhances production in rat aorta of 2-arachidonoyl glycerol, a hypotensive endocannabinoid. Eur J Pharmacol 362:R1–R3.

Meves H (1994) Modulation of ion channels by arachidonic acid. Prog Neurobiol 43:175–186.

Miller AS, Walker JM (1995) Effects of a cannabinoid on spontaneous and evoked neuronal activity in the substantia nigra pars reticulata. Eur J Pharmacol 279:179–185.

Moore SD, Madamba SG, Joëls M, Siggins GR (1988) Somatostatin augments the M-current in hippocampal neurons. Science 239:278–280.

Netzeband JG, Conroy SM, Parsons KL, Gruol DL (1999) Cannabinoids enhance NMDA-elicited Ca^{2+} signals in cerebellar granule neurons in culture. J Neurosci 19:8765–8777.

Pan XH, Ikeda SR, Lewis DL (1996) Rat brain cannabinoid receptor modulates N-type Ca^{2+} channels in a neuronal expression system. Mol Pharmacol 49:707–714.

Piomelli D (1994) Eicosanoids in synaptic transmission. Crit Rev Neurobiol 8:65–83.

Rinaldi-Carmona M, Barth F, Héaulme F, Shire D, Calandra B, Congy C, Martinez S, Marauni J, Néliat G, Caput D, Ferrara P, Soubrié P, Breliere JC, LeFur G (1994) SR 141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett 350:240–244.

Schmitt H, Meves H (1993) Protein kinase C as mediator of arachidonic acid-induced decrease of neuronal M current. Pflügers Arch 425:134–139.

Schweitzer P, Madamba SG, Siggins GR (1990) Arachidonic acid metabolites as mediators of somatostatin- induced increase of neuronal M-current. Nature 346:464–467.

Schweitzer P, Madamba SG, Champagnat J, Siggins GR (1993) Somatostatin inhibition of hippocampal CA1 pyramidal neurons: mediation by arachidonic acid and its metabolites. J Neurosci 13:2033–2049.

Shen MX, Thayer SA (1998) The cannabinoid agonist Win55,212–2 inhibits calcium channels by receptor-mediated and direct pathways in cultured rat hippocampal neurons. Brain Res 783:77–84.

Stella N, Schweitzer P, Piomelli D (1997) A second endogenous cannabinoid that modulates long-term potentiation. Nature 388:773–778.

Storm JF (1990) Potassium currents in hippocampal pyramidal cells. Prog Brain Res 83:161–187.

Sugiura T, Kodaka T, Kondo S, Nakane S, Kondo H, Waku K, Ishima Y, Watanabe K, Yamamoto I (1997) Is the cannabinoid CB1 receptor a 2-arachidonoylglycerol receptor? Structural requirements for triggering a Ca^{2+} transient in NG108–15 cells. J Biochem 122:890–895.

Szabo B, Dörner L, Pfreundtner C, Nörenberg W, Starke K (1998) Inhibition of GABAergic inhibitory postsynaptic currents by cannabinoids in rat corpus striatum. Neuroscience 85:395–403.

Tsou K, Brown S, Sañudo-Peña MC, Mackie K, Walker JM (1998) Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience 83:393–411.

Twitchell W, Brown S, Mackie K (1997) Cannabinoids inhibit N- and P/Q-type calcium channels in cultured rat hippocampal neurons. J Neurophysiol 78:43–50.

Wang HS, Pan ZM, Shi WM, Brown BS, Wymore RS, Cohen IS, Dixon JE, McKinnon D (1998) KCNQ2 and KCNQ3 potassium channel subunits: molecular correlates of the M-channel. Science 282:1890–1893.

Williams JH, Errington ML, Lynch MA, Bliss TVP (1989) Arachidonic acid induces a long-term activity-dependent enhancement of synaptic transmission in the hippocampus. Nature 341:739–742.

Xue BG, Belluzzi JD, Stein L (1993) *In vitro* reinforcement of hippocampal bursting by the cannabinoid receptor agonist (−)CP-55,940. Brain Res 626:272–277.