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Δ^9-Tetrahydrocannabinol Acts as a Partial Agonist to Modulate Glutamatergic Synaptic Transmission between Rat Hippocampal Neurons in Culture

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ABSTRACT

Δ^9-Tetrahydrocannabinol (Δ^9-THC) is the principal psychoactive ingredient in marijuana. We examined the effects of Δ^9-THC on glutamatergic synaptic transmission. Reducing the extracellular Mg^{++} concentration bathing rat hippocampal neurons in culture to 0.1 mM elicited a repetitive pattern of glutamatergic synaptic activity that produced intracellular Ca^{++} concentration spikes that were measured by indo-1-based microfluorimetry. Δ^9-THC produced a concentration-dependent inhibition of spike frequency with an EC_{50} of 20 ± 4 nM and a maximal inhibition of 41 ± 3%. Thus, Δ^9-THC was potent, but had low intrinsic activity. Δ^9-THC (100 nM) inhibition of spiking was reversed by 300 nM *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide (SR 141716), indicating that the inhibition was mediated by CB1 cannabinoid receptors. Δ^9-THC attenuated the inhibition produced by a full cannabinoid receptor agonist, (+)-[2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl](1-napthalenyl)methanone monomethanesulfonate (Win 55212-2), indicating that Δ^9-THC is a partial agonist. The effect of Δ^9-THC on synaptic currents was also studied. 6-Cyano-2,3-dihydroxy-7-niroquiinoxaline (CNQX)-sensitive excitatory postsynaptic currents were recorded from cells held at −70 mV in the whole-cell configuration of the patch-clamp and elicited by presynaptic stimulation with an extracellular electrode. Win 55212-2 and Δ^9-THC inhibited excitatory postsynaptic current (EPSC) amplitude by 96 ± 2% and 57 ± 4%, respectively. Excitatory postsynaptic current amplitude was reduced to 75 ± 5% in the presence of both drugs, demonstrating that Δ^9-THC is a partial agonist. The psychotropic effects of Δ^9-THC may result from inhibition of glutamatergic synaptic transmission. The modest physical dependence produced by Δ^9-THC as well as its lack of acute toxicity may be due to the ability of the drug to reduce, but not block, excitatory neurotransmission.

Δ^9-Tetrahydrocannabinol is the principal psychoactive ingredient in marijuana. Δ^9-THC produces euphoria, sedation, hypoactivity, hypothermia, hypotension and bradycardia (Abood and Martin, 1992; Lake et al., 1997). Dronabinol, Δ^9-THC in sesame oil, has been used clinically to stimulate appetite and reduce nausea in patients undergoing chemotherapy for cancer and AIDS (Plasse et al., 1991). Δ^9-THC also appears to have other useful clinical attributes including analgesic, antiglaucoma, and antiepileptic properties (Howlett, 1995; Adams and Martin, 1996).

The effects of Δ^9-THC are mediated by cannabinoid receptors that are distributed throughout the central nervous system (Herkenham et al., 1990; Tsou et al., 1998) and are present at high density on the presynaptic terminals of glutamatergic synapses (Twitchell et al., 1997). Cannabinoid receptors are members of the G-protein-coupled receptor family (Matsuda et al., 1990) and act via inhibitory G proteins (Childers et al., 1993) to activate K^+ channels (Deadwyler et al., 1993; Henry and Chavkin, 1995; Mackie et al., 1995) and inhibit Ca^{++} channels (Mackie and Hille, 1992; Twitchell et al., 1997; Shen and Thayer, 1998). The activation of these receptors by cannabimimetic drugs attenuates glutamatergic neurotransmission by acting presynaptically to inhibit the release of glutamate (Shen et al., 1996).

The cannabinoid neuromodulatory system exhibits an extensive pharmacology with several endogenous lipids proposed as ligands (Devane et al., 1992) as well as a number of synthetic cannabinoid (Johnson and Melvin, 1986) and aminoalkylindole (D'Ambra et al., 1992) derivatives that vary in potency, efficacy and stereoselectivity. In radioligand binding assays, compounds with affinities that range from subnano-

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ABBREVIATIONS: Δ^9-THC, Δ^9-tetrahydrocannabinol; NMDA, *N*-methyl-D-aspartate; Win55212–2 (R enantiomer), (+)-[2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl](1-napthalenyl)methanone monomethanesulfonate; SR141716, *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide; EPSC, excitatory postsynaptic current; CP55940, [1α,2β(R)5α]-(−)-5-(1,1-dimethylheptyl)-2-[5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]phenol.

molar to micromolar have been described (Devane et al., 1988; Herkenham et al., 1990). Some of the putative endogenous ligands as well as some of the cannabinoid derivatives behave as partial agonists in receptor mediated inhibition of Ca^{++} channels, G protein activation and synaptic transmission (Mackie et al., 1993; Pan et al., 1996; Shen et al., 1996; Sim et al., 1996b; Burkey et al., 1997a). In some behavioral assays, the maximal effects of Δ^9-THC were less than other cannabimimetic drugs, suggesting that it acted as a partial agonist (Compton et al., 1992). The stereoisomers of the aminoalkylindole Win55212-2 differ by over 100-fold in activity for inhibition of electrically stimulated mouse vas deferens (D'Ambra et al., 1992).

The effects of Δ^9-THC on excitatory synaptic transmission have not been described. In this report, we show that Δ^9-THC is a potent inhibitor of glutamatergic synaptic transmission, although it exhibits partial inhibition at maximal concentrations. The ability of this drug to reduce, but not block, excitatory neurotransmission explains some of its behavioral effects.

Materials and Methods

Materials were obtained from the following companies: Win55212-2 and 6-cyano-2,3-dihydroxy-7-nitroquinoxaline (CNQX), RBI, Natick, MA; *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide (SR141716), Sanofi Recherche, Montpellier Cedex, France; Δ^9-THC and all other reagents, Sigma Chemical Co., St. Louis, MO.

Rat hippocampal neurons were grown in primary culture as previously described (Wang et al., 1994) with minor modifications. Neurons dissociated from hippocampi of embryonic day 17 rats were plated as a droplet onto glass coverslips at an approximate density of 2.2×10^4 cells/cm^2 (5×10^4 cells/well). Cultures were grown without mitotic inhibitors for a minimum of 12 days before use.

Whole-cell currents were recorded with an Axopatch 200A patch-clamp amplifier and the BASIC-FASTLAB interface system (Indec Systems, Sunnyvale, CA). For recording EPSCs, pipettes (3–5 MΩ resistance) were pulled from borosilicate glass (Narashige USA, Inc. Greenvale, NY) and filled with a solution containing: K-gluconate, 130 mM; KCl, 10 mM; NaCl, 10 mM; 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid, 10 mM; HEPES, 10 mM; Glucose, 10 mM; MgATP, 5 mM; Na_2GTP, 0.3 mM; 300 mOsm/kg, adjusted to pH 7.2. The extracellular solution was composed of: NaCl, 140 mM; KCl, 5 mM; $CaCl_2$, 3 mM; $MgCl_2$, 6 mM; glucose, 5 mM; HEPES, 10 mM; bicuculline methchloride, 0.01 mM, and was adjusted to pH 7.4 with NaOH and to 315 mOsm/kg with sucrose. EPSCs were evoked with an extracellular bipolar concentric electrode placed next to the cell body of the presynaptic cell. The high $[Mg^{++}]_o$ reduced polysynaptic responses and isolated the non-*N*-methyl-D]-aspartate (NMDA) component of the synaptic response.

Kainate and NMDA-gated currents were recorded from cells held at −70 mV and elicited by a 15 sec bath application of agonist (100 μM) applied every 5 min. Kainate-evoked currents were recorded in the same solutions used to record EPSCs. For NMDA-evoked currents, the pipette was filled with $CsMeSO_3$, 125 mM; CsCl, 15 mM; $CaCl_2$,3 mM; 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid, 11 mM; HEPES, 20 mM; MgATP, 5 mM; Na_2GTP, 0.3 mM, pH 7.2 with CsOH, 300 mOsm/kg, and the external solution contained KCl, 5 mM; NaCl, 137 mM; $CaCl_2$, 1.3 mM; HEPES, 20 mM; glucose, 5 mM; glycine, 10 μM; strychnine, 2μM; bicuculline methchloride, 10 μM; CNQX, 10μM; and tetradotoxin, 0.1μM; pH 7.4 with NaOH, 315 mOsm/kg with sucrose. These currents were filtered at 20 Hz and sampled every 10 μs. Displayed currents were not corrected for leak.

$[Ca^{++}]_i$ was measured in single hippocampal neurons by indo-1-based microfluorimetry as described previously (Shen et al., 1996). Experiments were performed at room temperature in a recording chamber (Thayer et al., 1988) that was continuously perfused with buffer composed of the following : HEPES, 20 mM; NaCl, 137 mM; $CaCl_2$, 1.3 mM; $MgCl_2$, 0.1 mM; KCl, 5.0 mM; KH_2PO_4, 0.4 mM; Na_2HPO_4, 0.6 mM; $NaHCO_3$, 3.0 mM; glucose, 5.6 mM; and glycine, 0.01 mM; pH 7.45.

Data are presented as mean ± S.E.M. Statistical comparisons were made by Student's *t* test and analysis of variance (ANOVA) with Bonferoni's post-test.

Results

Reducing the $[Mg^{++}]_o$ in the media bathing hippocampal cultures to 0.1 mM elicits an intense pattern of $[Ca^{++}]_i$ spiking activity. Underlying each $[Ca^{++}]_i$ spike is an intense burst of action potentials. This electrical activity is driven by excitatory neurotransmission that is inhibited by antagonists of both NMDA and nonNMDA-type ionotropic glutamate receptors (McLeod et al., 1998). The frequency of $[Ca^{++}]_i$ spikes can be used as an index of glutamatergic synaptic activity. Indeed, we found that cannabinoid modulation of $[Ca^{++}]_i$ spiking was paralleled by similar modulation of synaptic currents (Shen et al., 1996). In this study, we used this method to study the effects of Δ^9-THC on excitatory neurotransmission. As shown in Fig. 1A, bathing hippocampal neurons in 0.1 mM $[Mg^{++}]_o$ produces a stable pattern of $[Ca^{++}]_i$ spikes. Application of 100 nM Δ^9-THC reduced $[Ca^{++}]_i$ spike frequency by 40%. Increasing the concentration of Δ^9-THC to 1 μM did not inhibit the spike frequency further, although application of the cannabimimetic Win55212-2, a drug we have shown previously to be a full agonist, completely blocked low $[Mg^{++}]_o$-induced $[Ca^{++}]_i$ spiking (Fig. 1B). A complete concentration-response curve was generated for Δ^9-THC-induced inhibition of $[Ca^{++}]_i$ spiking activity. These data are plotted with data from the full agonist Win55212-2 (Shen et al., 1996) in Fig. 4C. The slope factors, which in these experiments are equivalent to the Hill coefficients, were 1.3 ± 0.2 and 1.6 ± 0.3 for Δ^9-THC and Win55212-2, respectively, suggesting that Δ^9-THC activates a single class of noninteracting binding sites (De Lean et al., 1978). Win55212-2 inhibited completely low $[Mg^{++}]_o$-induced $[Ca^{++}]_i$ spiking with an EC_{50} of 2.7 ± 0.3 nM. The EC_{50} for Δ^9-THC was 20 ± 4 nM and the maximal inhibition was 41 ± 3%, indicating that in this system Δ^9-THC exhibited high potency, but rather modest efficacy.

The psychotropic effects of Δ^9-THC are mediated by CB1 cannabinoid receptors (Matsuda et al., 1990). We used the selective CB1 receptor antagonist, SR141716, to determine whether the inhibitory effect of Δ^9-THC on excitatory neurotransmission in hippocampal cultures was mediated by this receptor. As shown in Fig. 2A, 5 min pretreatment with 300 nM SR141716 completely prevented the effects of subsequent application of 100 nM Δ^9-THC. In the absence of antagonist, 100 nM Δ^9-THC inhibited the $[Ca^{++}]_i$ spiking frequency by 40 ± 5% (n = 4) (Fig. 1A). SR141716 alone did not significantly alter the basal spiking frequency (Fig. 2B). The high superfusion rate used in these experiments precludes drawing conclusions regarding inhibitory tone mediated by endogenous cannabinoid receptor agonists.

The modest efficacy of Δ^9-THC displayed in the concentration response curve suggested that this drug may act as a partial agonist on CB1 receptors to inhibit glutamatergic synaptic transmission. We tested this hypothesis by evaluat-

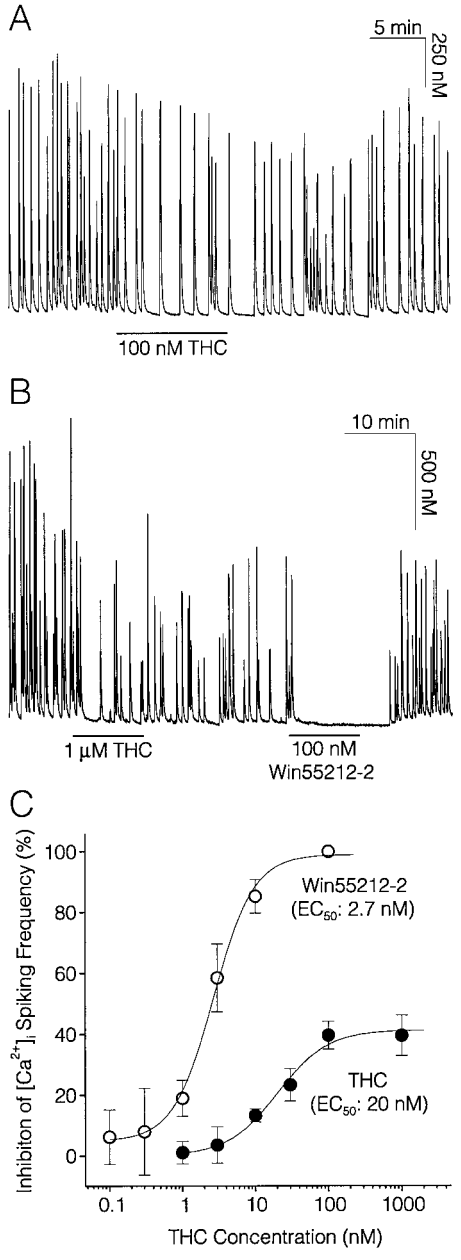

Fig. 1. Concentration-dependent inhibition of excitatory neurotransmission by Δ^9-THC. $[Ca^{++}]_i$ was recorded from single hippocampal neurons with indo-1-based microfluorimetry. $[Mg^{++}]_o$ was reduced to 0.1 mM for the entire recording and drugs were added by superfusion at the times indicated by the horizontal bars. A, representative trace shows that Δ^9-THC (THC, 100 nM) inhibited low $[Mg^{++}]_o$-induced $[Ca^{++}]_i$ spiking by 44%. B, increasing the Δ^9-THC concentration to 1 μM still produced partial inhibition of $[Ca^{++}]_i$ spiking. Subsequent application of 100 nM Win55212-2 to the same cell, after washout of the Δ^9-THC, completely blocked $[Ca^{++}]_i$ spiking. C, concentration-response curves show that both Δ^9-THC and Win55212-2 were potent inhibitors of the frequency of low $[Mg^{++}]_o$-induced $[Ca^{++}]_i$ spiking activity (EC_{50} = 20 ± 4 nM and 2.7 ± 0.3 nM, respectively). Win55212-2 data were replotted from our previous study (Fig. 4 of Shen et al., 1996) for the purposes of comparison. Data points represent three to six experiments such as those shown in A and B, and are expressed as mean ± S.E.M. Curves were fit by a logistic equation of the form percent Inhibition = $(I_{max})/(1+(X/EC_{50})^b)$, where X is the drug concentration, Im_{ax} is the percent Inhibition calculated for an "infinite" concentration, and b is a slope factor that determines the steepness of the curve. EC_{50} values were calculated by a nonlinear, least-squares curve fitting algorithm with Origin software (Microcal, Northampton, MA), and are expressed as mean ± S.E.M. I_{max} and b for Δ^9-THC were 41 ± 3% and 1.3 ± 0.2, respectively. I_{max} and b for Win55212-2 were 100% and 1.6 ± 0.3, respectively.

ing the ability of Δ^9-THC to reverse the effects of the full agonist Win55212-2. Application of 100 nM Win55212-2 to a cell in 0.1 mM $[Mg^{++}]_o$ completely blocked $[Ca^{++}]_i$ spiking (Fig. 3A). This inhibition was partially reversed by application of 100 nM Δ^9-THC. The low $[Mg^{++}]_o$-induced $[Ca^{++}]_i$ spiking frequency was inhibited by 64 ± 5% by the two drugs in combination. Δ^9-THC alone reduced spike frequency by 40 ± 5% (Fig. 3B). Clearly, Δ^9-THC has both agonist and antagonist properties.

The $[Ca^{++}]_i$ spiking activity induced by low $[Mg^{++}]_o$ results from the complex activity of a network of hippocampal neurons. In order to study glutamatergic synaptic activity in a more simple system, we recorded EPSCs by the whole-cell configuration of the patch-clamp technique. Synaptic currents were evoked by an extracellular concentric bipolar electrode placed near the soma of the presynaptic cell. The postsynaptic cell was voltage-clamped at −70 mV. To reduce polysynaptic responses, $[Mg^{++}]_o$ was increased to 6 mM. That, together with the omission of glycine from the media, also blocked NMDA-receptor-mediated currents. Thus, evoked EPSCs were completely blocked by CNQX (Fig. 4A) (n = 10). Δ^9-THC (100 nM) reduced EPSC amplitude by 57 ± 4% (n = 7;

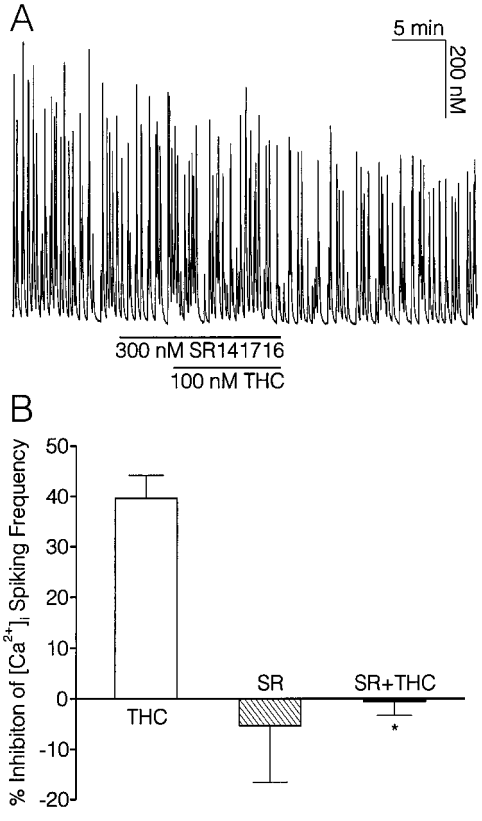

Fig. 2. Δ^9-THC inhibits 0.1 mM $[Mg^{++}]_o$-induced $[Ca^{++}]_i$ spiking via activation of the CB1 receptor. $[Ca^{++}]_i$ was recorded from single hippocampal neurons with indo-1-based microfluorimetry. 0.1 mM $[Mg^{++}]_o$-buffer was superfused throughout the recording and drugs were added by superfusion at the times indicated by the horizontal bars. A, representative trace shows that 5 min superfusion with the selective CB1 receptor antagonist, SR141716 (300 nM), prevented the effect of Δ^9-THC (100 nM) on low $[Mg^{++}]_o$-induced $[Ca^{++}]_i$ spiking. B, histogram shows that inhibition of $[Ca^{++}]_i$ spiking produced by 100 nM Δ^9-THC dropped from 40 ± 5% (n = 4) to –1 ± 3% (n = 3) in the presence of 300 nM SR141716 (SR). SR141716 (300 nM) itself increased the low $[Mg^{++}]_o$-induced $[Ca^{++}]_i$ spiking frequency by 5 ± 11% (n = 6). *, $p < .01$, SR + Δ^9-THC relative to Δ^9-THC alone (Student's t test).

$p < .001$ relative to control) in good agreement with the inhibition of low $[Mg^{++}]_o$-induced $[Ca^{++}]_i$ spiking activity produced by this drug (Fig. 4A and C). The full agonist Win55212-2 (100 nM) inhibited EPSC amplitude by 96 ± 2% ($n = 8$). Δ^9-THC partially reversed the inhibition produced by the full agonist as shown in Fig. 4B. Combined application of Win55212-2 and Δ^9-THC inhibited EPSC amplitude by 75 ± 5% ($n = 6$) (Fig. 4C) which was significantly different from that produced by Win55212-2 alone ($p < .001$).

We have shown previously that cannabimimetic drugs act presynaptically in this system to inhibit the release of glutamate (Shen et al., 1996). We explored the possibility that Δ^9-THC might have additional postsynaptic effects by studying the effects of this drug on whole-cell currents evoked by the direct activation of nonNMDA and NMDA currents (Figs. 4D and E, respectively). Kainate elicited a large inward current that was not significantly (paired t test) affected by 100 nM Δ^9-THC (1 ± 2% inhibition; $n = 6$). Kainate-evoked currents were blocked by 10 μM CNQX (95 ± 1% inhibition). NMDA also elicited large inward currents that were not significantly affected by 100 nM Δ^9-THC (6 ± 4% inhibition; $n = 4$). NMDA-evoked currents were blocked by 10 μM CGS19755 (85 ± 3% inhibition). These data are consistent with the idea that Δ^9-THC acts presynaptically to inhibit excitatory neurotransmission.

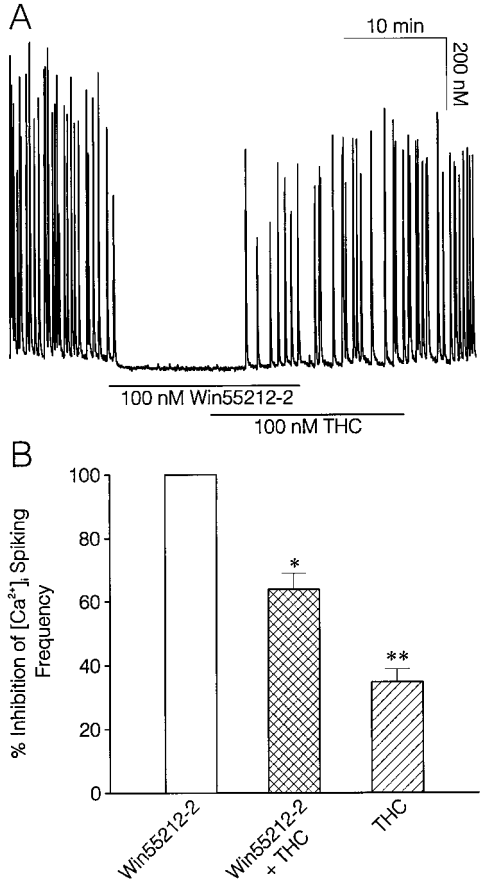

Fig. 3. Δ^9-THC acted as a partial agonist to inhibit low $[Mg^{++}]_o$-induced $[Ca^{++}]_i$ spiking. $[Ca^{++}]_i$ was recorded from single hippocampal neurons with indo-1-based microfluorimetry. 0.1 mM $[Mg^{++}]_o$-buffer was superfused throughout the recording and drugs were added by superfusion at the times indicated by the horizontal bars. A, Δ^9-THC (THC, 100 nM) partially reversed the inhibition of low $[Mg^{++}]_o$-induced $[Ca^{++}]_i$ spiking produced by 100 nM Win 55,212-2. B, histogram summarizes reduction in $[Ca^{++}]_i$ spike frequency produced by either Win 55,212-2 (100 nM) ($n = 3$) or Δ^9-THC (100 nM) ($n = 3$) alone or in combination ($n = 3$). *, $p < .01$; **, $p < .001$ relative to Win55212-2 alone (ANOVA with Bonferroni post test).

Discussion

Δ^9-THC inhibited glutamatergic synaptic transmission between rat hippocampal neurons grown in primary culture. This effect was observed as a reduction in the frequency of $[Ca^{++}]_i$ spikes evoked by reducing the $[Mg^{++}]_o$ to excite an entire synaptic network, or by inhibition of EPSCs elicited by direct stimulation of a presynaptic neuron. The inhibition was mediated by the CB1 cannabinoid receptor as indicated by antagonism by SR141716. The CB1 cannabinoid receptor is the predominant subtype in the brain (Tsou et al., 1998) and appears to mediate most of the behavioral effects of the cannabinoids (Matsuda et al., 1990). Δ^9-THC inhibited glutamatergic synaptic transmission with an EC_{50} of 20 nM, a value between the low nanomolar K_i values for Δ^9-THC displacement of [^{3}H]-[1α,2β(R)5α]-(−)-5-(1,1-dimethyl-heptyl)-2-[5-hydroxy-2-(3-hydroxypropyl)-cyclohexyl]phenol (CP55940) from brain membranes (Devane et al., 1988) and the submicromolar K_i values for displacement from brain slices (Herkenham et al., 1990). Δ^9-THC has been shown to stimulate [^{35}S]GTPγS binding with an EC_{50} in the 100 nM range (Sim et al., 1996a). Studies that used brain slice preparations tended to require higher concentrations of cannabimimetic drugs, possibly because of greater nonspecific binding of these lipophilic compounds to more intact preparations. We speculate that Δ^9-THC acted presynaptically to inhibit the release of glutamate similar to other cannabimimetic drugs we have tested in this system (Shen et al., 1996). Glutamatergic synaptic transmission in the hippocampus is essential for spatial learning tasks and cannabimimetic drugs have been shown to produce short term memory deficits in spatial learning paradigms (Lichtman and Martin, 1996), suggesting that the effects described here may account for some of the behavioral effects of Δ^9-THC.

Δ^9-THC exerted its effects on excitatory neurotransmission by acting as a partial agonist. This observation is consistent with tests of Δ^9-THC in behavioral paradigms (Compton et al., 1992) as well as cellular and molecular studies that have described partial agonists that act on cannabinoid receptors. Anandamide inhibition of Ca^{++} currents in N18 cells was of limited efficacy (Mackie et al., 1993) and CP55940 was found to inhibit Ca^{++} current as a partial agonist in sympathetic neurons expressing CB1 receptors (Pan et al., 1996). In a previous report from our laboratory, we showed that the synthetic cannabinoid CP55940 acted as a partial agonist to inhibit glutamate release (Shen et al., 1996). Sim et al. (1996a) and Burkey et al. (1997b) have shown that Δ^9-THC acts as a partial agonist to stimulate [^{35}S]GTPγS binding. Comparison of the efficacy for inhibition of glutamatergic synaptic activity with the structure of four cannabimimetic drugs, Δ^9-THC and CP55940 that acted as partial agonists and desacetyllevonantradol and Win55212-2 that acted as a full agonists, suggests that a free aliphatic side chain at position 3 on the phenolic ring and the absence of a secondary or tertiary amine in the structure may be common to cannabinoids with partial agonist properties. The relative efficacy of partial agonists is dependent on the stoichiometry of the components of the signal transduction pathway on which it acts (Weiss et al., 1996). The heterogeneous distribution of

G-protein subtypes might create local areas of varying sensitivity to cannabinoids (Breivogel et al., 1997). That some of the effects of Δ^9-THC might be mediated by antagonism of the endogenous ligand for the receptor is a more speculative possibility.

A withdrawal syndrome can be precipitated by administering an antagonist to rats chronically treated with high doses of Δ^9-THC (Tsou et al., 1995), although in humans, chronic Δ^9-THC use has not been associated with physical dependence (Hollister, 1986). Chronic administration of Δ^9-THC results in tolerance (Oviedo et al., 1993) and a desensitization of cannabinoid-mediated signaling processes (Sim et al., 1996a). Tolerance and desensitization might be more pronounced with drugs, such as Win55212-2, that have full agonist activity. In preliminary studies, we have found that Win55212-2 inhibition of low $[Mg^{++}]_o$-induced $[Ca^{++}]_i$ spiking desensitized during a 2 h exposure, in contrast to the inhibition produced by CP55940, a cannabimimetic with par-

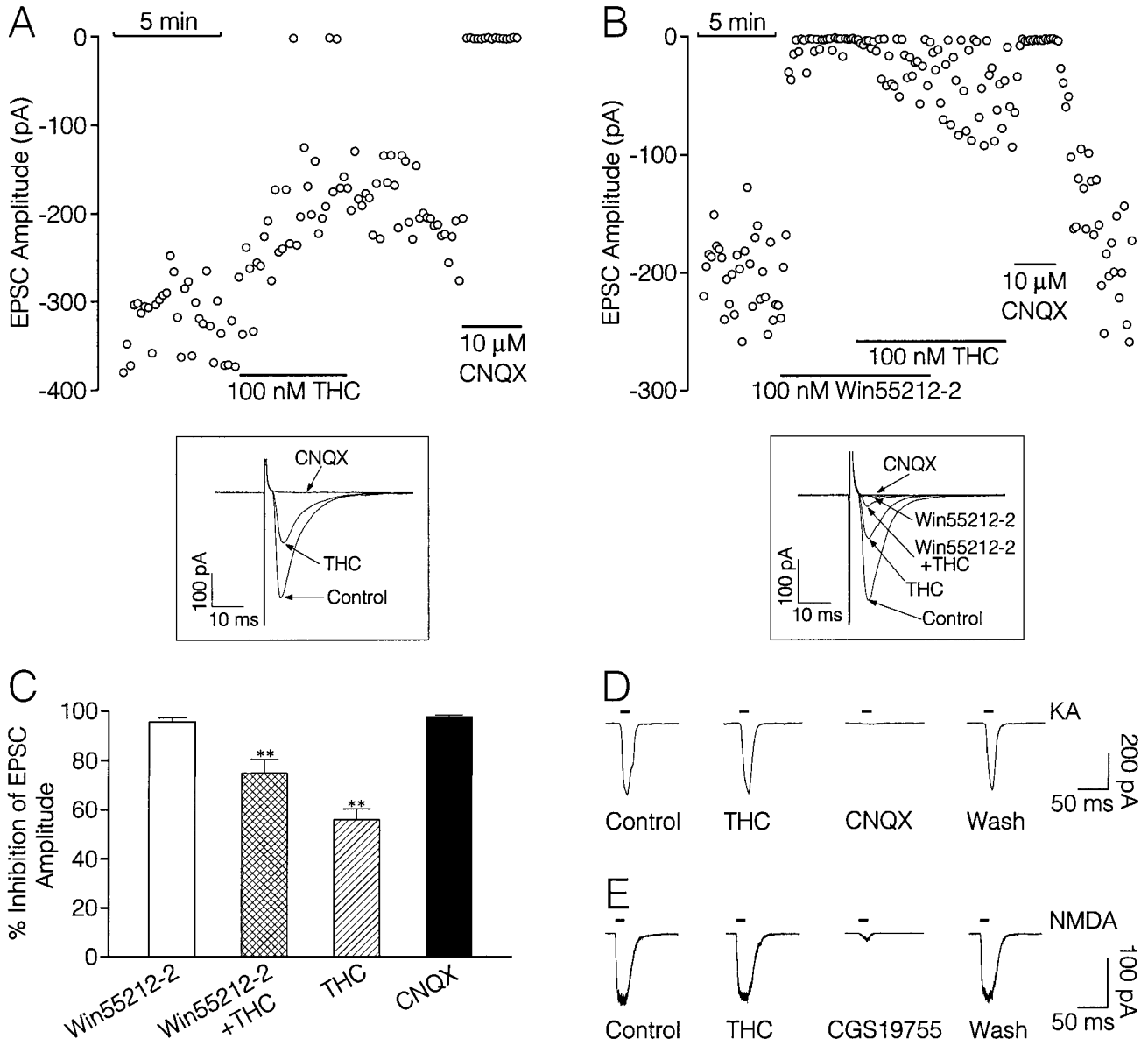

Fig. 4. Δ^9-THC is a partial agonist that modulates glutamatergic neurotransmission. A and B, EPSCs were evoked every 10 s by an extracellular electrode placed near the soma of the presynaptic cell and recorded from the postsynaptic cell held at −70 mV in the whole-cell configuration. Drugs were applied by superfusion as indicated by the horizontal bars. A, plot of EPSC amplitude versus time showing that 100 nM Δ^9-THC (THC) inhibited EPSC amplitude by 52%, whereas CNQX (10 μM) completely blocked EPSCs. B, Win55212-2 (100 nM) inhibited EPSC amplitude by 98%. Δ^9-THC (THC, 100 nM) partially reversed (to 85%) the inhibition of EPSC amplitude produced by Win55212-2. In the same cell, CNQX (10 μM) completely blocked EPSCs. Insets, EPSCs recorded during the experiments in A and B. Traces are mean EPSCs of twelve sweeps recorded during the last two min of each indicated treatment. C, histogram summarizes pooled data showing that 100 nM Win55212-2 inhibited EPSC amplitude by 96 ± 2% (n = 8). Δ^9-THC produced significantly less inhibition (57 ± 4%, n = 7). Δ^9-THC significantly attenuated the effects of Win55212-2. EPSC amplitude in the presence of both drugs was 75 ± 5% (n = 6) of control. 10 μM CNQX blocked glutamatergic synaptic transmission (n = 10). **, $p < .001$, relative to the inhibition produced by application of 100 nM Win 55212-2 alone (ANOVA with Bonferroni post test). D and E, whole-cell currents were recorded as described in *Materials and Methods*. Currents were evoked by superfusion with 100 μM kainate (D) or 100 μM NMDA (E) at the times indicated by the horizontal bars. Trace 2, Δ^9-THC (100 nM) applied 5 min prior and during agonist application did not affect the currents. Trace 3, 10 μM CNQX (D) and 10 μM CGS19755 (E) blocked the kainate- and NMDA-evoked currents, respectively. Trace 4, the effects of the antagonists were reversible.

tial agonist activity in our system, that produced a steady inhibition throughout the 2 h exposure (Shen and Thayer, 1996).

In summary, Δ^9-THC acts on CB1 receptors to inhibit glutamate-mediated synaptic transmission between cultured rat hippocampal neurons. In this in vitro system, Δ^9-THC was potent, but of modest efficacy, which may account for many of the behavioral effects of this drug.

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