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RAPID PUBLICATION

Cannabinoid Agonists Inhibit the Activation of 5-HT_3 Receptors in Rat Nodose Ganglion Neurons

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SUMMARY AND CONCLUSIONS

1. Effects of cannabinoid agonists on the serotonin (5-HT)$_3$ receptor-mediated current were investigated in rat nodose ganglion neurons. Anandamide, Win 55212–2, and CP55940 inhibited the 5-HT-induced current in a concentration dependent manner. IC_{50} values were 190, 310, and 94 nM for anandamide, Win 55212–2, and CP55940, respectively, and 1.6 μM for the nonpsychoactive enantiomer CP56667. This inhibition was slowly developing, noncompetitive, not dependent on membrane potential, and not affected by adenosine 3′,5′-cyclic monophosphate (cAMP) analogues, guanosine-5′-O-(2-thiodiphosphate) (GDP-β-S), and opioid receptor antagonist naltrexone. These data suggest that 5-HT_3 receptor ion-channel is a site acted upon by cannabinoid agonists in the nervous system, and the action of cannabinoid agonists on 5-HT_3 receptors may be a possible mechanism for some of the behavioral effects of cannabinoids, such as antiemesis and analgesia.

INTRODUCTION

Cannabinoids, the active constituents of marijuana, produce a great variety of behavioral effects in humans including euphoria, hallucinations, analgesia, and antiemesis (Dewey 1986; Howlett et al. 1990). The cellular mechanisms for these cannabinoid actions are poorly understood. It has been reported that cannabinoids inhibit 3′,5′-cyclic monophosphate (cAMP) production (Howlett et al. 1990) and the function of N-type calcium channels (Caufield and Brown 1992; Mackie and Hille 1992; Mackie et al. 1993). Recently, cannabinoid receptors were cloned (Matsuda et al. 1990; Munro et al. 1993) and anandamide, a brain constituent, was identified as an endogenous ligand for cannabinoid receptors (Devane et al. 1992).

The serotonin (5-HT)$_3$ receptor is a ligand-gated ion channel which is involved in mood, pain, emesis, some psychiatric disorders, and drug abuse (Greenshaw 1993). Independent studies from different laboratories showed that both cannabinoids and 5-HT_3 receptor antagonists can produce similar behavioral effects such as nonopioid analgesia, antiemesis in cancer patients and, involvement in mood and drug abuse (Dewey 1986; Greenshaw 1993; Howlett et al. 1990). These observations suggest that 5-HT_3 receptors may be involved in the actions of cannabinoids. The present study investigated the effects of cannabinoid agonists, anandamide, Win 55212–2, and CP55940 on the 5-HT_3 receptor-mediated current in rat nodose ganglion neurons.

METHODS

Nodose ganglion neurons were acutely isolated from adult Sprague-Dawley rats (Ikeda et al. 1986). 5-HT-induced current was recorded with the whole cell patch-clamp mode at −50 mV; temperature, 20–25°C. Neurons were superfused with extracellular solution contained (in mM) 150 NaCl, 5 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 10 D-glucose. Patch electrodes (2–5 MΩ) were filled with an internal solution containing (in mM) 140 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 11 ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA), 10 HEPES, 2 ATP, pH 7.4. Cannabinoids were first dissolved in dimethyl sulfoxide (DMSO); solutions containing cannabinoids were sonicated for 2–4 min before use. Drugs were diluted in external solution and applied through a fast perfusion system consisting of a series of fused silica tubes (200–300 μm) glued together and held by a micromanipulator. These tubes were connected to several different reservoirs containing either control or test solutions. The neuron under study was placed within 50 μm of the opening of these tubes and cell was exposed to the desired solution. The tested solution was removed by rapidly moving the perfusion system sidewise. CP55940 and CP56667 were kindly provided by Dr. M. Herkenham with the permission of Pfizer, and GDP-β-S was from Boehringer Mannheim. Other chemicals were purchased from Research Biochemical.

RESULTS

5-HT and the selective 5-HT_3 receptor agonist, 2-methyl-serotonin induced an inward current (Fig. 1*A*), which was sensitive to the specific 5-HT_3 receptor antagonist MDL72222 (20 nM, $n = 4$). Anandamide (10 nM to 3 μM) inhibited the current induced by 5-HT (Fig. 1*B*). The inhibition was slowly developing with a poor and incomplete recovery (Fig. 1, *B* and *D*). The inhibition was not dependent on membrane potential (Fig. 2*C*) and agonist concentrations (Fig. 2*D*). In the absence and presence of 350 nM anandamide, EC_{50} values of 5-HT were 2.3 and 2.5 μM, respectively, and the apparent Hill coefficients were 1.22 and 1.19. Similar effect was obtained with the synthetic cannabinoid agonists Win 55212–2 and CP55940 (Fig. 1, *C* and *D*; Fig. 2*B*). IC_{50} values for anandamide, Win 55212–2 and CP55940 were 190, 310, and 94 nM (Fig. 2, *A* and *B*), respectively, and the apparent Hill coefficients were 1.9, 1.2, and 1.7. IC_{50} value for CP56667, the less active enantiomer of CP55940, was 1.6 μM, which is 17 times higher than that of CP55940 (Fig. 2*B*).

Although extracellularly applied Sp-cAMP, the nonhydrolyzable analogue of cAMP, may affect desensitization

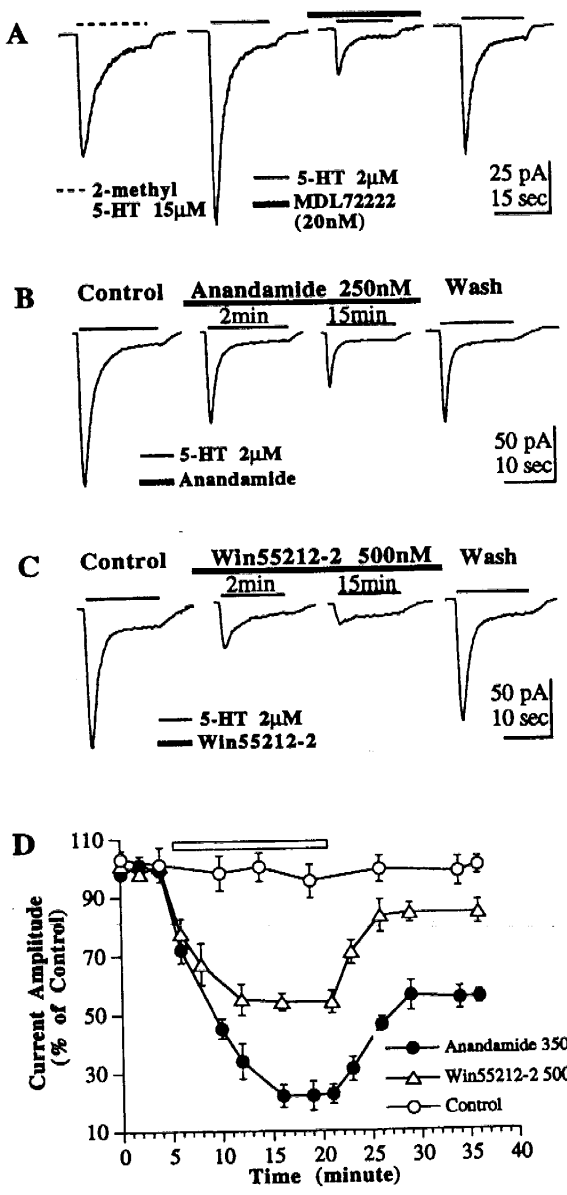

FIG. 1. Effects of cannabinoid agonists on the 5-HT induced current. *A*: currents induced by 5-HT (——) and the selective 5-HT_3 receptor agonist 2-methyl-5-HT (– – –). The current was blocked by MDL72222 (■). Anandamide (*B*, ■) and Win 55212–2 (*C*) inhibited the current induced by 2 μM 5-HT. Time (in min) after the introduction of cannabinoid agonists is indicated above traces. *D*: time course for the cannabinoids effects. Current was induced by 2 μM 5-HT. Application of anandamide (350 nM) or Win 55212–2 (500 nM) was indicated by a bar. Each point represents the average data from 4 to 9 cells.

of 5-HT-induced current (unpublished observations), intracellular application of Sp-cAMP and GDP-β-S, the nonhydrolyzable analogue of GTP, through the recording electrodes neither affected the 5-HT-induced current nor the action of anandamide on the current induced by 5-HT (Fig. 3, *B* and *C*). No significant difference was found ($P > 0.05$–0.1, Fig. 3*D*). Intracellular applications of 0.5 to 4 mM cAMP (Fan 1994) for 15 to 30 min had no effect on the 5-HT-induced current. Effects of Sp-cAMP on the 5-HT-induced current and the actions of cannabinoids are under further study. The action of anandamide was also

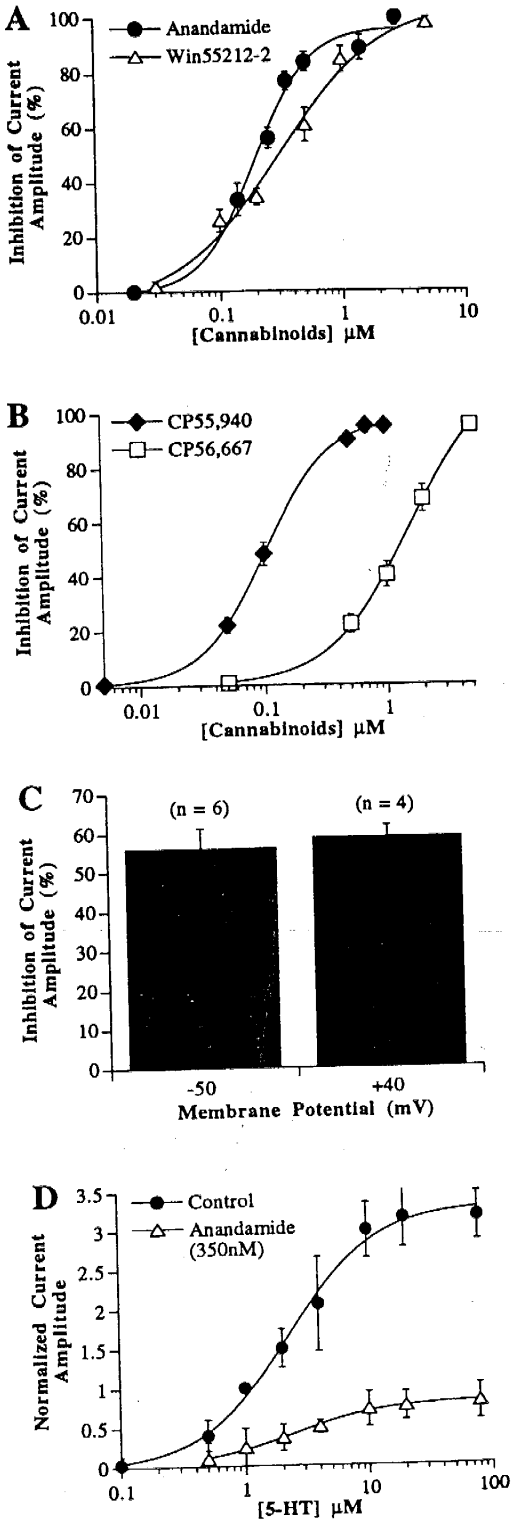

FIG. 2. Concentration-response curves of anandamide and Win 55212–2 (*A*), CP55940 and CP56667 (*B*). Currents in *A* and *B* were induced by 2 μM 5-HT. *C*: effect of 250 nM anandamide at membrane potential of −50 mV ($n = 6$) and +40 mV ($n = 4$). *D*: concentration-response curves of 5-HT in the absence and the presence of 350 nM anandamide. Currents were normalized to that by 1 μM 5-HT. Data were collected after 15-min application of anandamide. Each point represents the average data from 4–8 cells.

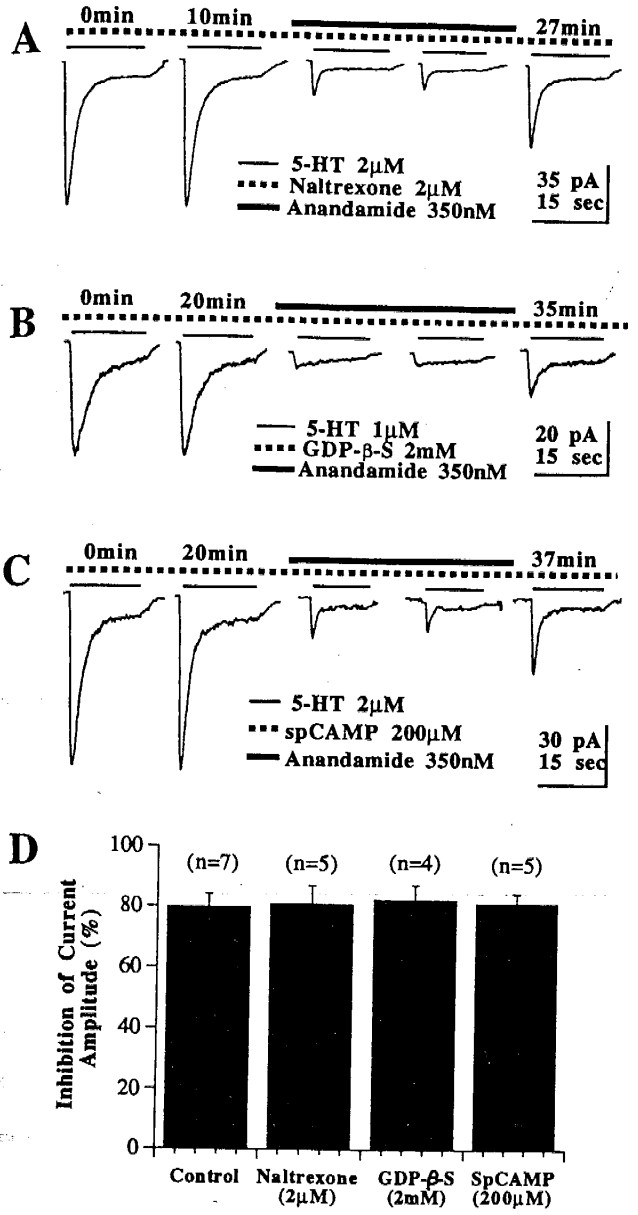

FIG. 3. Effects of naltrexone, GDP-β-S, and Sp-cAMP. Application of 2 μM naltrexone (A) and intracellular GDP-β-S (2 mM, B) or Sp-cAMP (200 μM, C) was indicated by dashed bars. Exposure to 5-HT and anandamide (350 nM) was indicated by thin and thick bars, respectively. Time (in min) after the introduction of naltrexone, GDP-β-S or Sp-cAMP is shown above traces. Anandamide was applied for 15 to 20 min. The average data for the effect of 350 nM anandamide is shown in D.

not affected by opioid antagonist, naltrexone (2 μM, n = 5, Fig. 3A).

DISCUSSION

The present study demonstrates that nanomolar concentrations of cannabinoid agonists inhibit the function of a neurotransmitter receptor. The inhibition was noncompetitive and not dependent on membrane potential. The IC_{50} values of cannabinoids on the 5-HT-induced current were higher than those on calcium current (Mackie and Hille 1992; Mackie et al. 1993) and those in most of the receptor binding studies

TABLE 1. *Potencies of cannabinoid agonists*

	Binding Affinity Ki, nM*	Inhibition of Adenylate Cyclase, Ki, nM*	Inhibitionn of 5-HT Current, IC_{50}, nM
Anandamide	52–90	540–1900	190
Win 55212-2	8.8–564	24–320	310
CP55,940	0.068–3.7	0.87–25	94
CP56,667	3.4–470	96.3–5000	1600

* Data for binding affinity and inhibition of adenylate cyclase were from Childers et al. 1994; Devane et al. 1992; Pertwee 1993; Vogel et al. 1993.

(Table 1). However, these values are comparable to the half maximal concentrations for the inhibition of cAMP production (Table 1) and that for the inhibition of vas deferentia contraction by anandamide (Devane et al. 1992). These data suggest that 5-HT$_3$ receptor is a possible site for the action of cannabinoid agonists.

Because the effect of anandamide on 5-HT$_3$ receptor-mediated current was not affected by naltrexone and cAMP analogues, it is unlikely that this effect of anandamide is through opioid receptors or cAMP mediated processes. The observation that GDP-β-S had no influence on the action of anandamide did not support the involvement of a G-protein in the present study. However, in some cases, GDP-β-S did not block G-protein-mediated responses. It is therefore premature to conclude whether a G-protein is involved in the present study.

It has been suggested that most of the behavioral effects of cannabinoids appear to be receptor mediated (Pertwee 1993). A much higher IC_{50} value of the less active enantiomer CP56667 (17 times of that of CP55940) suggests that hydrophobicity of these compounds is not a factor which determines their effects on the 5-HT-induced current. The observed difference in IC_{50} values reveals some stereospecificity but the involvement of cannabinoid receptors still requires further evidence.

The impairment of the function of a neurotransmitter receptor by cannabinoid agonists suggests that the related neurotransmission and modulation of transmitter release may also be affected. This observation also provides a possibility that some of the cannabinoid agonist-induced effects may be achieved by the inhibition of 5-HT$_3$ receptor function. The similar analgesic and antiemetic effects of cannabinoids and 5-HT$_3$ receptor antagonists may lead to a speculation that 5-HT$_3$ receptors may be involved in the cannabinoid-induced analgesia, antiemesis, and possibly other behavioral effects. To support this hypothesis, however, additional investigation is needed to establish a relationship between the effects caused by 5-HT$_3$ receptor antagonists and those induced by cannabinoids.

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