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Neuroscience Letters 301 (2001) 69–71

Neuroscience Letters

www.elsevier.com/locate/neulet

Activation of CB1 cannabinoid receptors in rat hippocampal slices inhibits potassium-evoked cholecystokinin release, a possible mechanism contributing to the spatial memory defects produced by cannabinoids

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Received 10 November 2000; received in revised form 15 December 2000; accepted 27 December 2000

Abstract

Cannabinoid use is known to disrupt learning and memory in a number of species. cholecystokinin (CCK) release and CCK receptors have been implicated in spatial memory processes in rodents. Rat hippocampal CCK interneurons express cannabinoid 1 receptors (CB1). The CB1 agonist *R*(+)WIN 55,212–2 (WIN+), at 1 and 10 μmol, strongly inhibited potassium-evoked CCK release from rat hippocampal slices, while the inactive isomer *S*(−)WIN,55,212–3 (WIN−) had no effect. CCK release from cerebral cortical slices was not altered by WIN+.

Keywords: Cholecystokinin; CB1; Cannabinoids; Spatial memory; WIN 55,212–2

Cannabinoids have been consumed for a long time for medical and recreational uses. They are known to cause euphoria, reduce anxiety, reduce pain, impair spatial memory and increase appetite [6]. An endogenous cannabinoid neurotransmitter or neuromodulator system consisting of two cannabinoid receptors and the endogenous ligands, anandamide (*N*-arachidonylethanolamine) and 2-arachidonylglycerol has been identified. CB1 and its splice variant CB1A are found predominantly in the brain, being very abundant in hippocampus, cerebellum and striatum. CB2 is present mainly in the periphery. Cannabinoids inhibit adenylate cyclase activity and reduce cholinergic, glutamatergic, γamino butyric acid (GABA)ergic and dopaminergic neurotransmission [1].

Cholecystokinin (CCK) is one of the most abundant peptides in the cortex and hippocampus [3]. CCK-positive interneurons in the rodent hippocampus contain GABA [9] and at least 86% of CB1 positive interneurons in the hippocampus express CCK, this represents 97% of all CCK-positive interneurons. Co-localization of CCK and CB1 receptors has been confirmed by two other groups [11,16]. The synthetic cannabinoid agonist WIN 55,212–2 (WIN+) decreased electrical field stimulation-induced ^{3}H GABA release from superfused rat hippocampal slices [10].

Activation of CB1 receptors may cause desynchronization of principal cells and disrupt theta rhythms resulting in disruption of spatial memory. In rodents, cannabinoids are known to inhibit LTP and to inhibit short-term and spatial memory [15]. Cannabinoids also disrupt learning in primates [17]. Mice lacking the CB1 receptor have enhanced memory [13] and LTP [4].

Release of CCK and CCK receptors have been implicated in spatial memory in rodents. CCK release in hippocampus has been shown to accompany acquisition but not performance of a spatial memory task in rats [14]. CCK 2 knockout mice have defective spatial memory in comparison to wild type mice [14]. CCK 1 receptor mutant rats are less able to perform a spatial memory task (radial arm maze) than normal rats [12]. It is possible that inhibition of CCK release by cannabinoids contributes to these defects in memory. This study was initiated to evaluate whether activation of the CB1 receptor on hippocampal CCK-positive interneurons would alter potassium-evoked CCK release.

CCK release methods follow published procedures [5,7]. Horizontal sections (225 μm) from the frontal cortex and hippocampus from 200–250 g male Sprague–Dawley rats (Charles River) were placed into small plastic baskets fabri-

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0304-3940/01/$ - see front matter
PII: S0304-3940(01)01591-9

cated from 1 ml pipette tips with Nitex mesh glued to the bottom. These baskets were placed in 7 ml flat bottom vials containing 0.75 ml Krebs–Ringer Bicarbonate buffer (KRB) and incubated at 37°C with constant bubbling with 95% O_2/5%CO_2 basal KRB contains 119 mM NaCl, 2.5 mM KCL, 1.3 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1 mM NaH2PO_4, 26.2 mM $NaHCO_3$ and 11.1 mM glucose was bubbled with 95%O_2/5%CO_2 to a final pH of 7.4.

The slices were subjected to three 20 min preincubation periods in basal KRB. A 2 min basal release period was followed by a 2 min stimulation period in KRB containing 40 mM potassium. In the high potassium KRB buffer, equimolar amounts of NaCl was replaced by KCl to maintain iso-osmotic conditions. For most experiments, six baskets of hippocampal slices were used. The drug to be tested was added to the solution in half of the baskets and vehicle to the others as control. The drug to be tested was added at the beginning of the third and final 20 min preincubation period and was included in the subsequent basal and stimulus buffers. The release media was assayed for CCK release by CCK RIA as previous described [3]. The ED_{50} of the assay under these conditions was 4–10 pg/ml. The basal release values from control and drug treated baskets were averaged and compared with Student's paired *t*-test. The basal release value of each basket was subtracted from its respective stimulated value and these corrected values for control and drug treated slices were expressed as percentage of the control value and compared with the Student's paired *t*-test. This was necessary because there was considerable variability in the release from different sets of slices. Basal release from hippocampal slices was between 0–1.6 pg/vial while stimulated release was from 1–20 pg/vial. Basal release from cerebral cortical slices was between 0 and 7 pg/vial while stimulated release from 5 to 100 pg/vial.

The drugs *R*(+)-[2,3-Dihydro-5-methyl-3-[(morpholininyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl)methanone mesylate (WIN +) and *S*(−)-[2,3-Dihydro-5-methyl-3-[(morpholininyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl)methanone mesylate (WIN−) were obtained from RBI-Sigma. They were dissolved in dimethyl sulfoxide (DMSO) and added at a final DMSO concentration of 0.2%. A similar amount of DMSO was added to control vials.

Treatment of rat hippocampal slices with the CB1 cannabinoid agonist WIN+ at 1 and 10 μmol concentrations did not alter basal CCK release but decreased potassium-stimulated release 37.4 and 40.3%, respectively (Fig. 1A,B). Treatment of rat hippocampal slices with the inactive isomer of the CB1 cannabinoid agonist WIN− at 1 μmol did not alter basal or potassium-stimulated release (Fig. 1C). CCK release was more variable in the presence of this drug but CCK release in its presence was not different than control release. Treatment of slices of frontal cortex with 10 μmol WIN+ had no effect on either basal or potassium-evoked release (Fig. 1D).

The CB1 agonist WIN+ at the same doses that inhibit LTP in rodents [15] and inhibits GABA release [10], significantly inhibits potassium-evoked CCK release in slices of the rat hippocampus. This effect was not observed with the inactive agonist and was not observed with the active agonist in cerebral cortical slices. CB1 receptors on GABA and CCK positive interneurons in the hippocampus that have previously been shown to inhibit GABA release [10], also strongly inhibits CCK release. This inhibition of CCK release is probably related to inhibition of adenylate cyclase as cyclic AMP is a major stimulator of CCK release in endocrine cells and brain slices [2].

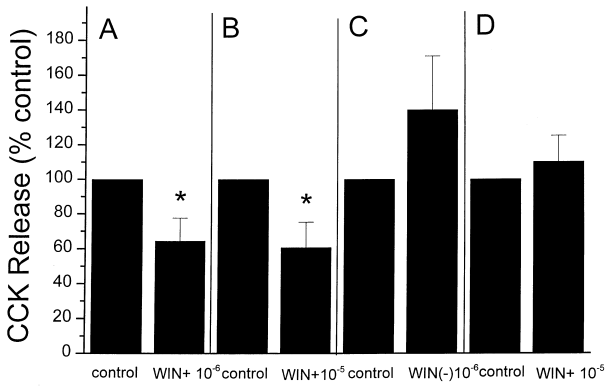

Fig. 1. Effect of WIN+ and WIN− on potassium-evoked CCK release from slices of hippocampus and cerebral cortex. The number in parentheses is the number of animals tested in each group. (A) One micromolar WiN+ in hippocampus (7); (B) 10 μmol WIN+ in hippocampus (6). (C) One micromolar WIN− in hippocampus (8); (D). 10 μmol WIN+ in cerebral cortex (3). The asterisk indicates that CCK release from drug treated slices differs from control, $P < 0.05$.

The maximal inhibition of CCK release by WIN+ at 10 μmol is 40% (see Fig. 1). That the inhibition of CCK release is not complete with WIN+ at this dose suggests that there are additional terminals that release CCK in response to the depolarization of high potassium that do not have CB1 receptors and whose release is unaffected by the agonist. The most likely source of this CCK release is dendrites or axon collaterals from CCK positive hippocampal cells which project to septum, mammilary bodies and the bed nucleus of the stria terminalis [8].

Previous studies have demonstrated that CCK is involved in learning and memory in a several species [14]. The ability of cannabinoids acting through CB1 receptors to inhibit CCK release may contribute to the learning and memory defects observed with cannabinoid use.

Cannabinoids also reduce anxiety, relieve pain and increase food intake. As CCK is known to cause anxiety, to antagonize opiate analgesia and to decrease food intake, it is conceivable that cannabinoids acting at CB1 receptors decrease CCK release and may be involved in effects of cannabinoids in other brain regions.

This work was supported in part by NS 18667, NS 31602 and DA12866.

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