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Short Communication

Type-1 Cannabinoid Receptor Activity During Alzheimer's Disease Progression

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Abstract. The activity of CB_1 cannabinoid receptors was studied in postmortem brain samples of Alzheimer's disease (AD) patients during clinical deterioration. CB_1 activity was higher at earlier AD stages in limited hippocampal areas and internal layers of frontal cortex, but a decrease was observed at the advanced stages. The pattern of modification appears to indicate initial hyperactivity of the endocannabinoid system in brain areas that lack classical histopathological markers at earlier stages of AD, indicating an attempt to compensate for the initial synaptic impairment, which is then surpassed by disease progression. These results suggest that initial CB_1 stimulation might have therapeutic relevance.

Keywords: Alzheimer's disease, cannabinoid receptors, functional autoradiography, G-protein, ligand binding

INTRODUCTION

The decline in synaptic function appears at early stages of Alzheimer's disease (AD), which correlates with cognitive dysfunction in AD patients [1, 2] at areas innervated by the cholinergic cells of the basal forebrain [3, 4]. The neuropathological markers, used for the classification of AD patients in different stages, have been found in subjects without dementia that may represent a preclinical stage of the illness [5].

The study of the endocannabinoid synapse is particularly interesting with regard to AD because cannabinoid receptor expression and other components of the endocannabinoid system have been found to be modified [6]. Reduction of CB_1 receptor density has been described in the hippocampus and caudate-putamen [7]. The localization of the cannabinoid receptors in the brain suggests its involvement in the modulation of learning and memory [8]. Some cannabinoid compounds are able to induce amnesia and memory deficits in mice [9] and regulate fear-conditioned memory [10]. In addition, increase in cannabinoid tone appears to induce neuronal survival [11]. The genetic deletion of CB_1 receptors induces neuronal loss in the hippocampus, accompanied by

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a decline in cognitive functions [12]. Therefore, the activity mediated by CB_1 receptors might regulate some cognitive functions and neuroprotective actions.

In the present study, we analyze the activity and density of CB_1 cannabinoid receptors in postmortem tissue from patients during AD progression.

MATERIALS AND METHODS

Brain tissue samples from 17 control cases and 36 AD patients were obtained from the tissue bank of the Hospital of Bellvitge, Barcelona. The AD patients were divided into three groups according to Braak's stages [13, 14], and were matched for age, postmortem delay, and freezing storage time.

Tissue sections were incubated with 0.04 nM guanosine [^{35}S]5′-O-[gamma-thio]triphosphate ([^{35}S]GTPγS). Agonist-stimulated binding was measured in the presence of the specific cannabinoid receptor agonist WIN55,212-2 (10^{-4} M), and consecutive slices were incubated with 3 nM [^{3}H]CP55,940 (more details in Supplementary Material).

Differences between groups of patients were analyzed using the Kruskal-Wallis non-parametric test followed by Dunn's *post-hoc* test. Correlations were applied to compare the [^{35}S]GTPγS with the [^{3}H]CP55,940 binding sites (Pearson's or Spearman's test).

RESULTS

In the present study, we used functional [^{35}S]GTPγS autoradiography to analyze the frontal cortex (Brodmann area 8), amygdala, basal forebrain (nucleus basalis of Meynert, nbM), striatum, hippocampus, and entorhinal cortex for CB_1-mediated activation of $G_{i/o}$ proteins in the presence of the cannabinoid agonist WIN55,212-2. We also employed [^{3}H]CP55,940 autoradiography to calculate the density of CB_1 receptors.

Analysis of the [^{35}S]GTPγS binding stimulated by WIN55,212-2 (Table 1) showed an upward trend during AD stages I-II of the functional CB_1 receptors at layer VI of frontal cortex.

The activity of CB_1 receptors was lower during AD stages V-VI than during stages I-II in the pyramidal and radiatum layers of the hippocampal CA1. The increased activity measured in the AD I-II group compared with the control group was partially responsible for this significant effect. This increase was statistically significant in the hilus of the dentate gyrus during stages I-II and decreased during stages V-VI. At stages III-IV, the CB_1 activity was similar to the control group in most of the hippocampal areas (Supplementary Fig. 1). The increased activity at stages I-II might be delaying the deterioration of CB_1-mediated activity. In contrast, in the lateral nucleus of the amygdala, CB_1-mediated activity decreased from the initial stages of the disease. In the nbM and striatal areas, CB_1 activity was not altered in AD.

We quantified the cannabinoid receptor density by measuring the specific labeling of the radioligand [^{3}H]CP55,940 (Table 2). The CB_1 cannabinoid receptors were upregulated in layer VI of the frontal cortex in patients with stage III-IV AD. In the hippocampus, CB_1 receptor density was altered in different hippocampal subfields in AD patients depending on the AD stage. Receptor density was increased relative to control cases mainly during AD stages III-IV. The density of receptors decreased to or fell below control levels at the most advanced stages (Supplementary Fig. 1). In the amygdala, CB_1 density was very low, and the measured densities of cannabinoid receptors were maintained during disease progression. Cannabinoid receptor density was not altered in the nbM of AD patients. In the caudate-putamen, the CB_1 receptor density increased during the initial stages of AD compared with control densities and a trend to decrease to control levels was observed during the next stages.

DISCUSSION

The aim of the present study was to analyze the status of the endocannabinoid system during AD progression. Patients were divided in three groups according to Braak's neuropathological stages (stages I-II, III-IV, and V-VI) [13, 15]. We observed regulation of the activation of the signaling cascade by $G_{i/o}$ proteins mediated through cannabinoid receptors during AD progression. The data obtained from the [^{3}H]CP55,940 autoradiography did not correlate with the WIN55,212-2-stimulated binding. Therefore, receptor density and receptor efficiency are modulated separately. The different lipid composition of the neuronal membranes can modulate the CB_1 activity [16].

The frontal cortex tissue sections from AD patients exhibit an apparent increase of the CB_1 activity in the AD I-II stages compared to AD III-IV. Previous studies that analyzed CB_1 receptor density and expression in cortical areas did not describe changes in AD [7, 17], but other binding and PET studies showed that CB_1 densities were reduced in frontal cortex [6,

Table 1
Autoradiographic densities for the specific binding of [^{35}S]GTPγS in human brain (nCi/g t.e.) stimulated by WIN55,212-2 in frontal cortex, hippocampus, and entorhinal cortex of control and AD patients

Brain area	Control	AD I-II	AD III-IV	AD V-VI
Frontal cortex	($n=4$)	($n=5$–8)	($n=6$)	
Layer I-III	45 ± 33	64 ± 23	53 ± 23	–
Layer IV	20 ± 15	71 ± 26	53 ± 33	–
Layer V	59 ± 55	130 ± 49	82 ± 47	–
Layer VI	78 ± 68	212 ± 430	125 ± 60	–
Hippocampus	($n=4$–7)	($n=3$–5)	($n=3$–4)	($n=4$–8)
CA1				
Lacunosum moleculare	101 ± 34	238 ± 76	29 ± 37	129 ± 17
Oriens	134 ± 58	197 ± 43	–	100 ± 56
Pyramidal	296 ± 82	512 ± 109	–	194 ± 25^{*,d}
Radiatum	166 ± 31	321 ± 70	–	154 ± 33^{*,d}
CA3				
Lacunosum moleculare	176 ± 113	484 ± 120	323 ± 152	140 ± 19
Oriens	147 ± 39	227 ± 65	–	91 ± 34
Pyramidal	252 ± 88	436 ± 156	–	162 ± 31
Radiatum	218 ± 53	451 ± 122	–	93 ± 37
Dentate gyrus				
Granular	80 ± 23	201 ± 75	27 ± 33	0 ± 6^{*,d}
Hilus	87 ± 22	236 ± 83^{*,a}	15 ± 29	11 ± 6^{*,d}
Molecular	258 ± 61	523 ± 142	171 ± 62	80 ± 32^{*,d}
Subiculum				
Lacunosum moleculare	110 ± 42	208 ± 72	42 ± 40	90 ± 36
Oriens	101 ± 54	219 ± 32	64 ± 28	73 ± 25
Pyramidal	301 ± 44	525 ± 123	205 ± 69	152 ± 36^{*,c,d}
Radiatum	236 ± 89	324 ± 162	111 ± 56	138 ± 49
Entorhinal cortex	($n=4$–7)	($n=3$–5)	($n=3$–4)	($n=4$–8)
Layer I	165 ± 35	417 ± 239	116 ± 109	108 ± 56
Layer II-III	218 ± 55	364 ± 226	175 ± 136	105 ± 29
Layer IV-VI	169 ± 45	407 ± 203	173 ± 107	88 ± 35
Amygdala	($n=4$)	($n=8$–12)	($n=7$–9)	
Lateral nucleus	321 ± 159	−68 ± 51^{*,a}	58 ± 88^{*,b}	–
Basal nucleus (magnocellular)	44 ± 46	90 ± 32	55 ± 15	–
Basal forebrain	($n=7$)	($n=12$)	($n=9$)	($n=5$)
Nucleus basalis (Meynert)	138 ± 38	173 ± 66	97 ± 55	113 ± 64
Striatum	($n=6$)	($n=13$)	($n=11$)	($n=9$)
Caudate-putamen	277 ± 97	197 ± 48	170 ± 45	169 ± 50

Data are mean ± SEM values. (n): number of cases used. (–): less than three samples available. The p values were calculated by the Kruskal-Wallis non-parametric test followed by Dunn's test. [a]AD I-II versus control, [b]AD III-IV versus control, [c]AD V-VI versus control, [d]AD I-II versus AD V-VI. $^{*}p<0.05$.

18, 19]. The increase of CB_1 receptor activity that we observed during the initial stages of AD might indicate a neuroprotective action mediated by endocannabinoids in response to initial neural damage, which has been extensively reviewed [20]. Analysis of the [^{3}H]CP55,940 binding sites during disease progression revealed a significant increase of CB_1 receptor density at layer VI of the frontal cortex in the AD III-IV patient group. The results indicate that the regulation of CB_1-mediated activity precedes the increase in receptor density. The increased efficiency could be less metabolically costly to the cell than the increased availability of new receptors.

Recent studies have reported a reduction in the enzyme responsible for the synthesis of anandamide in the cortex of AD patients [21]. The regulation of the CB_1 density and activity that we describe at cortex might be a compensatory mechanism to balance the anandamide signaling.

In a previous study CB_1 receptor density has been found decreased at the hippocampus in AD patients [7]. But when we analyzed it in detail during the progression of the disease, the CB_1 activation was greater during AD stages I-II, decreased to levels similar to the control group during AD stages III-IV, and continued to fall below control group levels during AD stages V-VI. These effects were significant at the pyramidal layers and the dentate gyrus, areas in which the cannabinoid receptors are more densely located at synaptic terminals. Therefore, the increase of activity

Table 2
Autoradiographic densities for the specific binding of [^{3}H]CP55,940 in the human brain samples from control and AD patients (fmol/mg)

Brain area	Controls	AD I-II	AD III-IV	AD V-VI
Frontal cortex	($n=9$)	($n=9$–10)	($n=6$)	
Layer I-III	57.8 ± 13.9	56.2 ± 7.8	67.1 ± 12.8	–
Layer IV	59.7 ± 16.4	51.1 ± 5.4	69.4 ± 10.9	–
Layer V	54.6 ± 13.1	60.4 ± 7.8	64.9 ± 8.6	–
Layer VI	50.4 ± 9.8	68.5 ± 6.1	$91.2 \pm 8.6^{*,b}$	–
Hippocampus				
CA1	($n=4$–6)	($n=4$–7)	($n=5$)	($n=4$–5)
Lacunosum moleculare	40.6 ± 5.1	48.4 ± 6.5	51.4 ± 8.4	42.8 ± 3.2
Oriens	40.6 ± 8	42.2 ± 3.1	39.3 ± 4.6	43.9 ± 5.4
Pyramidal	64.6 ± 9.6	84.1 ± 10	87.5 ± 15.9	$48.1 \pm 2.1^{*,d}$
Radiatum	42.5 ± 5.9	46.8 ± 5.7	55.7 ± 9.6	44.4 ± 4.9
CA3	($n=6$–7)	($n=7$–8)	($n=6$)	($n=7$)
Lacunosum moleculare	44.2 ± 6.7	53.9 ± 5.2	65.8 ± 5.5	$40.5 \pm 3.3^{*,d}$
Oriens	42.6 ± 5.1	42.2 ± 3.7	51.9 ± 7.6	44.5 ± 4.7
Pyramidal	68.3 ± 7.3	88.4 ± 6.2	98.3 ± 9.6	$58.1 \pm 3.5^{*,c,d}$
Radiatum	41.6 ± 5.0	47.0 ± 8.9	$59.6 \pm 6.2^{*,b}$	44.9 ± 4.5
Dentate gyrus	($n=4$–6)	($n=6$)	($n=7$)	($n=4$–5)
Granular	80.2 ± 10.1	95.8 ± 3.5	99.5 ± 8.5	$70.8 \pm 8.5^{*,c}$
Hilus	64.3 ± 9.9	67.7 ± 6.3	74.6 ± 6.6	65.2 ± 5.6
Subiculum	($n=5$–7)	($n=6$–7)	($n=5$–6)	($n=6$)
Lacunosum moleculare	44.6 ± 6.5	46.4 ± 7.5	64.8 ± 7.1	$36.7 \pm 1.2^{*,d}$
Oriens	45.5 ± 6.3	38.3 ± 4.6	55.9 ± 6.3	42.4 ± 2.7
Pyramidal	59.4 ± 6.5	$101.1 \pm 2.7^{*,a}$	107.3 ± 13.7	$49.1 \pm 4.2^{*,d}$
Radiatum	44.8 ± 6.2	59.3 ± 8.6	72.4 ± 5.6	$36.3 \pm 1.1^{*,d}$
Entorhinal cortex	($n=4$)	($n=3$)	($n=3$)	($n=3$)
Layer I	71.3 ± 17	55.1 ± 15.8	60.9 ± 0.6	58.7 ± 14.9
Layer II-III	53.0 ± 10.8	73.2 ± 5.9	$94.5 \pm 3.9^{*,b}$	$67.8 \pm 22.1^{*,d}$
Layer IV-VI	48.5 ± 9.1	63.5 ± 9.5	70.0 ± 3.2	$60.5 \pm 14^{*,d}$
Amygdala	($n=3$–4)	($n=6$–9)	($n=4$–5)	
Lateral nucleus	7.1 ± 1.9	5.3 ± 0.9	4.3 ± 0.9	–
Basal nucleus (magnocellular)	3.9 ± 0.5	4.5 ± 0.4	3.5 ± 0.8	–
Basal forebrain	($n=4$)	($n=6$)	($n=4$)	
Nucleus basalis (Meynert)	47.1 ± 4.7	40.1 ± 0.9	41.5 ± 8.0	–
Striatum	($n=5$)	($n=12$)	($n=8$)	($n=5$)
Caudate-putamen	62.7 ± 8.6	$94.7 \pm 4.8^{*a}$	75.4 ± 6.9	$66.4 \pm 4.5^{*,c}$

Data are mean ± SEM values. (n): number of cases used. (–): less than 3 samples available. The p values were calculated by the Kruskal-Wallis non-parametric test followed by Dunn's test. [a]AD I-II versus control, [b]AD III-IV versus control, [c]AD I-II versus AD V-VI, [d]AD III-IV versus *AD* V-VI. $^*p<0.05$.

during AD stages I-II might be an initial response to neural impairment.

Moreover, an increase of monoacylglycerol lipase activity has been described in AD stages V-VI that might contribute to the accumulation of 2-arachidonoyl glycerol, resulting in synaptic failure with the consequent downregulation of CB_1 receptors [22]. However, immunohistochemical assays have described no changes in CB_1 receptors [23].

We observed that the regulation on CB_1 densities was delayed during the advance of AD compared with the modulation of CB_1 activity. The [^{3}H]CP55,940 binding sites were decreased at later stages in the pyramidal layers of different hippocampal areas and the inner layers of the entorhinal cortex.

The nbM cholinergic cells innervate the amygdala, where the functional CB_1 receptors decreased in the lateral nucleus of the amygdala in AD patients. In contrast, the number and expression of cannabinoid receptors in the human amygdala are low [24, 7]. We also observed low CB_1 receptor density in the amygdala, which was conserved in AD patients. This finding suggests that the down-regulation of cannabinoid activity is not indicating modulation in receptor density. On the contrary, CB_1 activity in the striatal area was conserved in AD patients, but CB_1 density was high and was increased at the initial AD stages, continuing to a reduction with the progression of the disease, which might be caused by the loss of afferents from areas such as the globus pallidus and the substantia nigra, where a reduction of CB_1 of receptors had been reported [7]. Although the incidence of co-morbidity with parkinsonism is frequent in AD patients, we were unaware if this was the case in any

of the cases included in the present study. In addition, maintained levels of CB_1 cannabinoid receptors have been described in Parkinson's disease [25].

In summary, CB_1 receptors were more efficient in the patients at the earlier AD stages, specifically in hippocampal areas. These regulations on CB_1 signaling might precede to the accumulation of the neuropathological markers of the AD in specific brain areas. On the contrary, at the most advanced stages of AD, CB_1 efficacy diminished in both the hippocampus and the frontal cortex. The modulation of CB_1 density follows the same pattern, but occurs later in the course of AD.

The initial hyperactivity of the endocannabinoid system accounts for the possible compensation of synaptic impairment; however, the intimate and unknown cause of AD continues with neurodegeneration and determines a loss of CB_1 synapses. CB_1 stimulation might have therapeutic relevance during the initial and moderate stages of AD.

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SUPPLEMENTARY MATERIAL

Supplementary tables are available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-140492.

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