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Brief Communication

Increase of cannabinoid CB₁ receptor density in the hippocampus of streptozotocin-induced diabetic rats

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Abstract

In the hippocampus, impaired neurophysiology, compromised neurogenesis, and eventually apoptosis accompany cognitive deficits in insulinopenic (type-1) diabetes (T1D). The underlying pathological mechanisms remain to be defined. The hippocampus has a high density of the cannabinoid type 1 receptor (CB₁R), which has been shown to control several brain functions affected by diabetes, such as synaptic plasticity, glucose utilisation, memory consolidation and cognition, and maturation and survival of hippocampal neurons. However, the role of this receptor has not been investigated yet in diabetic encephalopathy. We report now that in the streptozotocin animal model of T1D, the hippocampal densities of CB₁R protein and of specific CB₁R binding sites are significantly increased both in the nerve terminals and in total membranes (changes between 13% and 38%), whereas CB₁R mRNA expression is decreased by 25%, suggesting that CB₁Rs might play a role in diabetic encephalopathy.

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Diabetes mellitus is the most common metabolic disorder in man. It affects a plethora of tissues and organs, including the brain. "Diabetic encephalopathy" encompasses characteristic biochemical, electrophysiological, morphological and cognitive deficits in diabetic patients (for review see Trudeau et al., 2004). T1D rats display a similar cognitive impairment and display compromised long-term potentiation (LTP) and marked changes in the density of pre- and postsynaptic synaptic markers, decreased cell proliferation, and apoptosis have all been observed in certain cortical regions (Chabot et al., 1997; Grillo et al., 2005; Jackson-Guilford et al., 2000; Li et al., 2002). However, the etiology of diabetic encephalopathy is still ill-defined.

CB₁Rs are ubiquitously expressed in the brain, where they are mostly located presynaptically (although also located postsynaptically) in a wide range of neurons (for review see Mackie, 2005). In particular, they are highly abundant in the human and the rodent hippocampus (Katona et al., 1999, 2000; Degroot et al., 2006; for review see Mackie, 2005). Activation of presynaptic hippocampal CB₁Rs diminishes the release of GABA (Katona et al., 1999, 2000), glutamate (Kawamura et al., 2006), dopamine and acetylcholine (Degroot et al., 2006). These neurochemical findings may underlie the ability of CB₁R activation to control cognition and memory consolidation (Hampson and Deadwyler, 1999). Apart from their role in controlling neurotransmitter release, CB1Rs also control ion channels and intracellular signaling pathways that are linked to apoptotic processes (for review see Guzmán et al., 2001). CB₁R activation also can promote adult hippocampal neurogenesis (Jiang et al., 2005), and regulate subtype-selective interneuron

Abbreviations: CB₁R, CB₁ cannabinoid receptor; SDS, sodium dodecyl sulphate; STZ, streptozotocin; T1D, insulinopenic (type-1) diabetes; T2D, type-2 diabetes; LTP, long-term potentiation; QRT-PCR, quantitative real-time polymerase chain reaction assay.

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migration and specification (Berghuis et al., 2005). Of particular interest is the finding that in several brain areas including the hippocampus, CB₁R has been shown to modulate glucose utilisation (Pontieri et al., 1999; Freedland et al., 2003). In other words, CB₁Rs are intricately involved in a wide spectrum of physiological and pathological mechanisms that are affected in diabetic encephalopathy. Thus, we aimed to test whether the density and the expression of CB₁Rs receptor is changed in a classic animal model of T1D.

Animals were handled according with the EU guidelines for use of experimental animals (86/609/EEC). Diabetes was induced in 8-week-old male Wistar rats (Harlan Ibérica, Barcelona, Spain) with i.p. injection of streptozotocin (STZ, 65 mg/kg), dissolved in citrate buffer (10 mM, pH 4.5). Blood glucose levels were measured with a glucometer (Elite, Bayer-Portugal). Age-matched, vehicle-injected control rats were maintained in the same conditions, i.e. with food and water ad libitum. 30 days after the STZ treatment, rats were decapitated under halothane anaesthesia. Both hippocampi were rapidly dissected on ice and total membranes from the hippocampus were prepared as previously described (Köfalvi et al., 2005; Duarte et al., 2006). Briefly, the hippocampal tissue from each rat was homogenized at 4 °C in sucrose solution (0.32 M, containing 10 mM HEPES, 1 mM EDTA and 1 mg/ml BSA, pH 7.4), then centrifuged at $3000 \times g$ for 10 min at 4 °C. The supernatant was centrifuged at 14,000×g for 20 min at 4 °C, and an aliquot was resuspended in 5% (w/v) sodium dodecyl sulphate (SDS) solution. To obtain nerve terminal-enriched (synaptosomal) membranes, the remainder of the supernatant was resuspended in 1 ml of a 45% (v/v) Percoll solution made up in a Krebs solution (in mM: NaCl 140, KCl 5, HEPES 10, EDTA 1, glucose 1, pH 7.4). After centrifugation at $14,000 \times g$ (for 2 min at 4 °C), the top layer was washed in 1 ml Krebs solution and resuspended in 5% (w/v) SDS solution. Western blotting was performed as previously described (Köfalvi et al., 2005; Duarte et al., 2006). Briefly, protein concentration was determined and each sample was diluted with 5 volumes of SDS-PAGE buffer containing 30% (v/v) glycerol, 0.6 M dithiothreitol, 10% (w/v) SDS and 375 mM Tris-HCl, pH 6.8, boiled at 95 °C for 5 min and incubated for 2 h at 37 °C. Three different dilutions of the samples and pre-stained molecular weight markers (Amersham, UK) were loaded into polyacrylamide gels (7.5%), separated according to protein molecular weight by SDS-PAGE under reducing conditions, and then electro-transferred to polyvinylidene difluoride membranes (0.45 µm, Amersham). After blocking for 2 h at room temperature with 5% milk in Trisbuffered saline (pH 7.6; containing 0.1% Tween 20 (TBS-T)), membranes were incubated overnight at 4 °C with the L-15 rabbit anti-CB₁R antibody, directed against the last 15 amino acids of the rat CB₁R (1:500 dilution). The selectivity of the antibody has been confirmed in total membrane preparations, prepared from the brain of CB₁R null-mutant mice (Ledent et al., 1999) and of CD-1 wild-type mice (Fig. 1). After washing, membranes were incubated with alkaline phosphatase-conjugated anti-rabbit secondary antibody (Amersham, 1:10,000). We also confirmed that the secondary antibody did not yield any signal in the absence of the primary antibody. After three 20-min

washes in TBS-T with 0.5% milk, membranes were incubated with Enhanced Chemi-Fluorescence reagent (Amersham) for 5 min and then digitalized with a VersaDoc 3000 (Bio-Rad. USA), and densitometrically analyzed using the Quantity One (Bio-Rad) software package. Membranes were then stripped and re-probed with an anti- α -tubulin antibody (1:3000; Zymed Laboratories) to confirm that equal amounts of protein were applied to the gels. Radioligand binding assays were performed essentially identically to a previous study (Thomas et al., 1998). Briefly, hippocampal (either total or nerve terminal-enriched) membranes were resuspended in the incubation buffer (50 mM Tris, 2 mM MgCl₂, 1 mM EGTA, pH 7.4). A saturation curve was constructed using 5 different concentrations (0.25–10 nM) of the CB₁R receptor antagonist [³H]SR141716A (Amersham, 42.0 Ci/mmol). Binding was carried out for 1 h at room temperature (23-25 °C) with 32-51 µg of protein in a final volume of 200 µl of the incubation buffer. Specific binding was determined by subtraction of the non-specific binding, which was measured in the presence of 10 µM AM251 (Tocris), another selective CB₁R antagonist. All binding assays were performed in duplicate. The binding reactions were stopped by rapid vacuum filtration through glass fiber filters (GF/C filters; Whatman) using a 24-well Brandel harvester, followed by washing with 5 ml of incubation buffer containing 0.5% (w/v) BSA. The filters were then placed in scintillation vials with 4 ml of scintillation liquid (Packard Ultima Gold). The specific binding derived from these saturation experiments was fitted by non-linear regression to a one binding site equation, using the Raphson-Newton method, performed with a commercial software (GraphPad), to determine the binding parameters (dissociation constant, K_D; and maximal number of binding sites, B_{max}). For quantitative real-time PCR (QRT-PCR) analysis, we began by extracting total RNA from hippocampal tissue with MagNA Lyser Instrument and MagNA Pure Compact RNA Isolation kit (Roche, Portugal), according to the manufacturer's instructions. Reverse transcription for firststrand cDNA synthesis from each sample was performed using random hexamer primer with the Transcriptor First Strand cDNA Synthesis kit (Roche) according to manufacturer's instructions. Resulting cDNAs were used as template for realtime PCR, which was carried out on LightCycler instrument (Roche) using the FastStart DNA Master SYBR Green I kit (Roche), and CB₁R mRNA expression was calculated relative to B-actin mRNA expression. The following primers (obtained from Tib MolBiol, Germany) were used (accession numbers from NCBI database in parenthesis): CB₁R (NM_012784): forward 5'-AGA CCT CCT CTA CGT GGG CTC G -3', reverse 5'-GTA CAG CGA TGG CCA GCT GCT G-3', which originates a 314-bp product (Hansson et al., 2007); and β -actin (V01217): forward 5'-AAG TCC CTC ACC CTC CCA AAA G-3', reverse 5'-AAG CAA TGC TGT CAC CTT CCC-3', originating a 97-bp product (Peinnequin et al., 2004). Quantification was carried out based on standard curves run simultaneously with the test samples. The CB_1R and β -actin standards were generated by conventional PCR amplification. The PCR products were run in a 3% agarose gel electrophoresis to verify fragment size and the absence of other contaminating



Fig. 1. (A) Representative Western blots comparing the CB₁R immunoreactivity (\sim 53 kDa band) in total hippocampal membranes (upper left panel) and nerve terminal-enriched membranes (upper right panel) from control and STZ-treated rats. In all Western blotting evaluations, we confirmed that identical amounts of protein were loaded in control and test samples by evaluating α -tubulin immunoreactivity, as shown in the top panels. Middle panel: CB₁R immunoreactivity is absent in the membrane preparation (40 µg protein loaded) of the CB₁R knockout mouse but a specific band is present at \sim 55 kDa in the membrane preparation from the wildtype (WT) CD-1 mouse, confirming the selectivity of the antibody. Lower left and right panels: densities of the CB₁R at each protein concentration. Each data point represents the mean±SEM of CB₁R immunoreactivity from six different control rats (open symbols) and six STZ-treated rats (filled symbols), at three different protein loads, ensuring that there is indeed a constant increase of CB₁R immunoreactivity in hippocampal membrane preparations of rats, 30 days after STZ treatment. **P*<0.05 control vs. treated. (B) Maximal specific binding (*B*_{max}) of the selective CB₁R antagonist [³H]SR141716A in rats 30 days after vehicle or STZ injection. Nonspecific binding was determined in the presence of another selective CB₁R antagonist, AM251 (10 µM). Bars represent mean±SEM from four different rats, run in duplicate; **P*<0.05. (C) Expression of CB₁R mRNA in rats 30 days after vehicle or STZ injection. CB₁R mRNA levels (copies/µl) were determined with QRT-PCR and normalized to the level of β-actin mRNA. Bars represent mean±SEM from seven different rats, run in triplicate; **P*<0.05.

fragments, quantified by 260 nm absorbance, and serially diluted to produce the standard curve $(10^0 \text{ to } 10^8 \text{ copies/}\mu\text{l})$. Each real-time PCR reaction was run in triplicate and contained 2 µl of cDNA template, 0.3 µM of each primer, and 3 or 3.5 mM MgCl₂ (for CB₁R or β-actin, respectively), in a reaction volume of 20 µl. Cycling parameters were (for CB₁R or β-actin, respectively): 95 °C for 10 min to activate DNA polymerase, followed by 40 or 45 cycles at 95 °C for 10 s, annealing temperature of 62 °C for 10 s or 61 °C for 5 s, and a final extension step at 72 °C for 14 s or 4 s, in which fluorescence was acquired. Melting curve analysis was performed to ensure that only a single product was amplified. Data are presented as mean±SEM (or as mean and 95% confidence interval for K_D values). Student's *t*-test was used to compare data and significance was considered to be reached when P < 0.05.

STZ destroys pancreatic β -cells hampering insulin release (Gai et al., 2004; Szkudelski, 2001), a key feature of T1D. Accordingly, blood analysis showed that STZ-treated rats developed sustained hyperglycemia at days 3 and 30 (Table 1) following STZ treatment. Furthermore, STZ-treated rats failed to gain body weight (Table 1). As illustrated in Fig. 1A, STZtreatment significantly increased CB₁R immunoreactivity in

Table 1
Body weight and glycemia of the rats used in the experiments before and after
the induction of diabetes ($n=6$ for each condition)

	Weight (g)		Glycemia (mg/dl)	
	Control	STZ-treated	Control	STZ-treated
Before treatment 3 days after treatment 1 month after treatment	225±14 n.d. 327±21*	231±11 221±7 208±15**	91±4 n.d. 93±1	96±6 460±14* 448±24*,**

n.d.: not determined.

* P < 0.01, different from before treatment.

** P<0.01, different from control.

total membranes (13.0 \pm 2.5%, n=6, P<0.05), as well as in nerve terminal-enriched membranes (14.8 \pm 3.7%, n=6, P < 0.05), derived from the hippocampus. These data correspond to the average of the mean differences found for each tested amount of protein. Previously, we observed that changes in protein density in membranes may not automatically mean similar changes in the density of binding sites (Duarte et al., 2006). Thus, we analysed CB_1R binding in total and nerve terminal-enriched membranes. We found a 38% increase in the $B_{\rm max}$ (a measure of the density of the receptor) in the total membranes of the hippocampus of the STZ-treated rats (n=4, P < 0.05, Fig. 1B), and an 18% increase in the nerve terminalenriched membranes (n=4, P<0.05, Fig. 1B). These different percentage (i.e. relative) increases of CB₁R density might correspond to similar absolute amounts of CB_1Rs since the B_{max} of [³H]SR141716A was 86% greater (P < 0.05) in nerve terminal-enriched than in total hippocampal membranes (Fig. 1B, black bars). In parallel, we observed that the $K_{\rm D}$ values (a measure of the affinity of a ligand for a receptor) tend to increase upon STZ treatment, although this did not reach the level of significance (from 1.61 nM [95% confidence interval: 1.21-2.01 nM] to 2.71 nM [1.80-3.62 nM] in total membranes; and from 1.06 nM [0.89-1.24 nM] to 1.59 nM [1.10-2.08 nM] in nerve terminal-enriched membranes). To test if increased CB₁R density in hippocampal membranes of STZ-treated rats is due to an altered mRNA expression, we performed QRT-PCR in total hippocampal membranes obtained from vehicle-injected and STZ-treated rats, 30 days after injection. Melting curve analysis confirmed that one single product has been obtained. Intriguingly, the expression of CB₁R mRNA (normalized to β-actin from the same sample) is significantly decreased by 25% (n=7, P < 0.05, Fig. 1C) in the STZ-treated animals.

Involvement of the CB₁R in metabolic disorders such as obesity and non-insulin-dependent diabetes (T2D) has attracted much attention in the last decade. Deletion of CB₁Rs leads to leanness and resistance to diet-induced obesity (Cota et al., 2003), which anticipates a major role for CB₁Rs in controlling T2D, given that obesity is a major risk factor for T2D. CB₁Rs can also control insulin levels (Juan-Pico et al., 2006), Matias et al., 2006), and CB₁R blockade can decrease hyperinsulinemia in obese subjects (Gelfand and Cannon, 2006). Furthermore, obese and/or hyperglycemic T2D patients exhibit higher endocannabinoid concentrations in serum and visceral fat (Matias et al., 2006). Accordingly, therapy with the CB₁R antagonist SR141716A (introduced to the European market under the name of Acomplia) is associated with weight loss, favourable changes in serum lipid levels, improved glycemic control in prediabetic and T2D patients, and decreased hyperinsulinemia in obese subjects (Gelfand and Cannon, 2006; Matias et al., 2006). However, to date, no report has suggested that CB₁Rs might play a role in T1D. In our T1D model, using STZ, we show now for the first time that CB₁R density is increased both in the total and in the nerve terminal-enriched membrane fraction in the rat hippocampus. This increased density of presynaptic CB₁Rs may reflect an elevated neuromodulator power for the CB₁R. In the hippocampus, endogenous activators of the CB₁R (i.e. endocannabinoids such as anandamide or 2-arachidonoyl glycerol) are released upon postsynaptic activation and then travel back to the presynaptic site to activate presynaptic CB₁Rs, inhibiting transmitter release (for review see Freund et al., 2003). This socalled retrograde transmission participates in both short and long forms of synaptic plasticity thought to be key elements of cognitive behaviour. LTP is impaired in STZ-induced diabetic animals (for review see Trudeau et al., 2004), which also display an impairment of Ca²⁺-dependent regulation of postsynaptic AMPA receptors (Chabot et al., 1997). Since endocannabinoids can facilitate the induction of LTP in the hippocampus via presynaptic blockade of GABAergic neurotransmission (Carlson et al., 2002) the upregulation of presynaptic CB_1Rs may contribute to restoring normal LTP functions. Alternatively, the increased CB₁Rs density may be an adaptive response to counterbalance impaired postsynaptic Ca²⁺ level regulation and AMPA functions that result in a diminished endocannabinoid release. Opposing to these, CB₁R activation can restrict LTP via presynaptic CB₁R activation in glutamatergic terminals (Slanina et al., 2005). Clearly, further functional studies will be required to explore the modification of CB₁R signalling in GABAergic and glutamatergic synapses in the hippocampus of STZ-treated rats to pinpoint the exact functional relevance of this modified density of CB₁Rs. It is of interest to note that we previously found that other G protein-coupled receptors (adenosine receptors) also undergo changes in their density after STZ treatment (Duarte et al., 2006). However, as for CB₁Rs, only functional studies will allow establishing if these changes in adenosine receptors are compensatory or part of the pathophysiological mechanisms in T1D. The observed increase in CB_1R density in total membranes upon STZ treatment suggests that there is a broader modification of CB₁R function in T1D. In fact, total membranes include a minority of synaptic component (<3%) and a majority of non-synaptic as well as intracellular membranes. Elevated CB₁R density in these fractions may reflect a compensatory role for CB₁Rs not only in synaptic plasticity but also in metabolic control, cell survival and neurogenesis. Indeed, CB₁Rs, forming heterodimers with TrkB NGF receptors, are responsible for correct migration and axonal arborization of certain neurons (Berghuis et al., 2005). The level of phosphorylated (active) Akt is also increased in the STZ model of diabetes, with a concomitant increase in levels of phosphorylated (inactive) glycogen synthase kinase 3ß (GSK3 β) (Clodfelder-Miller et al., 2005). Since CB₁Rs can activate the survival factor Akt (Gómez del Pulgar et al., 2002), and thus indirectly inhibit GSK3B, they can promote survival, as

well as dendritic arborization, which are normally controlled by the active form of GSK3ß (for review see Kalkman, 2006). Controlling glucose utilisation of hippocampal neurons (Pontieri et al., 1999; Freedland et al., 2003) is another aspect whereby CB₁Rs may either worsen or improve neuronal functions and survival chances in the absence of insulin, i.e. in T1D. Finally, the hypothesis that CB₁Rs might control glia-related functions, such as neuroinflammation, should not be excluded given that total membranes also include glial membranes. One striking aspect of our study was the observed disparity between changes in the expression and the density of CB₁Rs after STZ treatment. This suggests either a modification of protein translation, as was reported to occur in the hippocampus after traumatic brain injury (Chen et al., in press), where activation of a key rate-limiting translational pathway (the mTOR pathway) is observed; or alternatively a modification of the half-life of CB₁Rs that might be related to oxidative stress found in the hippocampus of STZinjected rats (Ates et al., 2006).

In conclusion, the elevated density of the CB_1R protein and CB_1R binding sites may represent a compensatory or a pathophysiological process. This change can either counteract or contribute to pathophysiological, structural and eventually, cognitive abnormalities, which involve synaptic deficits, hampered cell proliferation and apoptosis upon sustained hyperglycemia. Therefore, this study prompts the need to explore if the CB_1R antagonist SR141716A, which is now a therapeutically used drug, would worsen or improve neuropathological consequences of T1D.

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References

- Ates, O., Yucel, N., Cayli, S.R., Altinoz, E., Yologlu, S., Kocak, A., Cakir, C.O., Turkoz, Y., 2006. Neuroprotective effect of etomidate in the central nervous system of streptozotocin-induced diabetic rats. Neurochem. Res. 31, 777–783.
- Berghuis, P., Dobszay, M.B., Wang, X., Spano, S., Ledda, F., Sousa, K.M., Schulte, G., Ernfors, P., Mackie, K., Paratcha, G., Hurd, Y.L., Harkány, T., 2005. Endocannabinoids regulate interneuron migration and morphogenesis by transactivating the TrkB receptor. Proc. Natl. Acad. Sci. U. S. A. 102, 19115–19120.
- Carlson, G., Wang, Y., Alger, B.E., 2002. Endocannabinoids facilitate the induction of LTP in the hippocampus. Nat. Neurosci. 5, 723–724.
- Chabot, C., Massicotte, G., Milot, M., Trudeau, F., Gagne, J., 1997. Impaired modulation of AMPA receptors by calcium-dependent processes in streptozotocin-induced diabetic rats. Brain Res. 768, 249–256.
- Chen, S., Atkins, C.M., Liu, C.L., Alonso, O.F., Dietrich, W.D., Hu, B.R., in press. Alterations in mammalian target of rapamycin signaling pathways after traumatic brain injury. J. Cereb. Blood Flow Metab. (Electronic publication ahead of print).
- Clodfelder-Miller, B., De Sarno, P., Zmijewska, A.A., Song, L., Jope, R.S.,

2005. Physiological and pathological changes in glucose regulate brain Akt and glycogen synthase kinase-3. J. Biol. Chem. 280, 39723–39731.

- Cota, D., Marsicano, G., Tschop, M., Grubler, Y., Flachskamm, C., Schubert, M., Auer, D., Yassouridis, A., Thone-Reineke, C., Ortmann, S., Tomassoni, F., Cervino, C., Nisoli, E., Linthorst, A.C., Pasquali, R., Lutz, B., Stalla, G.K., Pagotto, U., 2003. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. J. Clin. Invest. 112, 423–431.
- Degroot, A., Köfalvi, A., Wade, M.R., Davis, R.J., Rodrigues, R.J., Rebola, N., Cunha, R.A., Nomikos, G.G., 2006. CB₁ receptor antagonism increases hippocampal acetylcholine release: site and mechanism of action. Mol. Pharmacol. 70, 1236–1245.
- Duarte, J.M.N., Oliveira, C.R., Ambrósio, A.F., Cunha, R.A., 2006. Modification of adenosine A₁ and A_{2A} receptor density in the hippocampus of streptozotocin-induced diabetic rats. Neurochem. Int. 48, 144–150.
- Freedland, C.S., Whitlow, C.T., Smith, H.R., Porrino, L.J., 2003. Functional consequences of the acute administration of the cannabinoid receptor antagonist, SR141716A, in cannabinoid-naive and -tolerant animals: a quantitative 2-[¹⁴C]deoxyglucose study. Brain Res. 962, 169–179.
- Freund, T.F., Katona, I., Piomelli, D., 2003. Role of endogenous cannabinoids in synaptic signaling. Physiol. Rev. 83, 1017–1066.
- Gai, W., Schott-Ohly, P., Schulte im Walde, S., Gleichmann, H., 2004. Differential target molecules for toxicity induced by streptozotocin and alloxan in pancreatic islets of mice in vitro. Exp. Clin. Endocrinol. Diabetes 112, 29–37.
- Gelfand, E.V., Cannon, C.P., 2006. Rimonabant: a cannabinoid receptor type 1 blocker for management of multiple cardiometabolic risk factors. J. Am. Coll. Cardiol. 47, 1919–1926.
- Gómez del Pulgar, T., De Ceballos, M.L., Guzmán, M., Velasco, G., 2002. Cannabinoids protect astrocytes from ceramide-induced apoptosis through the phosphatidylinositol 3-kinase/protein kinase B pathway. J. Biol. Chem. 277, 36527–36533.
- Grillo, C.A., Piroli, G.G., Wood, G.E., Reznikov, L.R., McEwen, B.S., Reagan, L.P., 2005. Immunocytochemical analysis of synaptic proteins provides new insights into diabetes-mediated plasticity in the rat hippocampus. Neuroscience 136, 477–486.
- Guzmán, M., Sánchez, C., Galve-Roperh, I., 2001. Control of the cell survival/ death decision by cannabinoids. J. Mol. Med. 78, 613–625.
- Hampson, R.E., Deadwyler, S.A., 1999. Cannabinoids, hippocampal function and memory. Life Sci. 65, 715–723.
- Hansson, A.C., Bermúdez-Silva, F.J., Malinen, H., Hyytia, P., Sanchez-Vera, I., Rimondini, R., Rodriguez de Fonseca, F., Kunos, G., Sommer, W.H., Heilig, M., 2007. Genetic impairment of frontocortical endocannabinoid degradation and high alcohol preference. Neuropsychopharmacology 32, 117–126.
- Jackson-Guilford, J., Leander, J.D., Nisenbaum, L.K., 2000. The effect of streptozotocin-induced diabetes on cell proliferation in the rat dentate gyrus. Neurosci. Lett. 293, 91–94.
- Jiang, W., Zhang, Y., Xiao, L., Van Cleemput, J., Ji, S.P., Bai, G., Zhang, X., 2005. Cannabinoids promote embryonic and adult hippocampus neurogenesis and produce anxiolytic- and antidepressant-like effects. J. Clin. Invest. 115, 3104–3116.
- Juan-Pico, P., Fuentes, E., Javier Bermudez-Silva, F., Javier Diaz-Molina, F., Ripoll, C., Rodriguez de Fonseca, F., Nadal, A., 2006. Cannabinoid receptors regulate Ca²⁺ signals and insulin secretion in pancreatic beta-cell. Cell Calcium 39, 155–162.
- Kalkman, H.O., 2006. The role of the phosphatidylinositide 3-kinase-protein kinase B pathway in schizophrenia. Pharmacol. Ther. 110, 117–134.
- Katona, I., Sperlágh, B., Sík, A., Köfalvi, A., Vizi, E.S., Mackie, K., Freund, T.F., 1999. Presynaptically located CB₁ cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. J. Neurosci. 19, 4544–4558.
- Katona, I., Sperlágh, B., Maglóczky, Z., Sántha, E., Köfalvi, A., Czirják, S., Mackie, K., Vizi, E.S., Freund, T.F., 2000. GABAergic interneurons are the targets of cannabinoid actions in the human hippocampus. Neuroscience 100, 797–804.
- Kawamura, Y., Fukaya, M., Maejima, T., Yoshida, T., Miura, E., Watanabe, M., Ohno-Shosaku, T., Kano, M., 2006. The CB₁ cannabinoid receptor is the

major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. J. Neurosci. 26, 2991–3001.

- Köfalvi, A., Rodrigues, R.J., Ledent, C., Mackie, K., Vizi, E.S., Cunha, R.A., Sperlágh, B., 2005. Involvement of cannabinoid receptors in the regulation of neurotransmitter release in the rodent striatum: a combined immunochemical and pharmacological analysis. J. Neurosci. 25, 2874–2884.
- Ledent, C., Valverde, O., Cossu, G., Petitet, F., Aubert, J.F., Beslot, F., Bohme, G.A., Imperato, A., Pedrazzini, T., Roques, B.P., Vassart, G., Fratta, W., Parmentier, M., 1999. Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB₁ receptor knockout mice. Science 283, 401–404.
- Li, Z.G., Zhang, W., Grunberger, G., Sima, A.A.F., 2002. Hippocampal neuronal apoptosis in type 1 diabetes. Brain Res. 946, 221–231.
- Mackie, K., 2005. Distribution of cannabinoid receptors in the central and peripheral nervous system. Handb. Exp. Pharmacol. 168, 299–325.
- Matias, I., Gonthier, M.P., Orlando, P., Martiadis, V., De Petrocellis, L., Cervino, C., Petrosino, S., Hoareau, L., Festy, F., Pasquali, R., Roche, R., Maj, M., Pagotto, U., Monteleone, P., Di Marzo, V., 2006. Regulation, function, and dysregulation of endocannabinoids in models of adipose and beta-pancreatic

cells and in obesity and hyperglycemia. J. Clin. Endocrinol. Metab. 91, 3171-3180.

- Peinnequin, A., Mouret, C., Birot, O., Alonso, A., Mathieu, J., Clarencon, D., Agay, D., Chancerelle, Y., Multon, E., 2004. Rat pro-inflammatory cytokine and cytokine related mRNA quantification by real-time polymerase chain reaction using SYBR green. BMC Immunol. 5 (3).
- Pontieri, F.E., Conti, G., Zocchi, A., Fieschi, C., Orzi, F., 1999. Metabolic mapping of the effects of WIN 55212-2 intravenous administration in the rat. Neuropsychopharmacology 21, 773–776.
- Thomas, B.F., Gilliam, A.F., Burch, D.F., Roche, M.J., Seltzman, H.H., 1998. Comparative receptor binding analyses of cannabinoid agonists and antagonists. J. Pharmacol. Exp. Ther. 285, 285–292.
- Slanina, K.A., Roberto, M., Schweitzer, P., 2005. Endocannabinoids restrict hippocampal long-term potentiation via CB₁. Neuropharmacology 49, 660–668.
- Szkudelski, T., 2001. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol. Res. 50, 537–546.
- Trudeau, F., Gagnon, S., Massicotte, G., 2004. Hippocampal synaptic plasticity and glutamate receptor regulation: influences of diabetes mellitus. Eur. J. Pharmacol. 490, 177–186.