

**Summary of the Ph.D. thesis**

**New Directions in Receptor Research;  
Receptor Selectivity and Promiscuity**

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## INTRODUCTION

G-protein-coupled receptors (GPCRs), the largest class of cell-surface receptors, are one of the major targets for many current and emerging drugs. Recent developments indicate novel levels of regulations of GPCRs functioning, such as cross-talk at the level of signaling, constitutive activity and oligomerization of GPCRs. The regulations of GPCRs at multiple levels cause emergence of complexity and specificity of GPCRs targeting.

Cannabinoid CB<sub>1</sub> (CB<sub>1</sub>) receptors are the most abundant GPCRs in the brain, with levels ten-fold higher than those of other GPCRs. The CB<sub>1</sub> receptor displays a significant level of constitutive activity, either in non-neuronal cells or in neurons. Increasing number of evidences indicate that the CB<sub>1</sub> receptors show different levels of interaction with other receptor types. Particularly, the CB<sub>1</sub> receptor system shares several features with both the μ-opioid (MOR) and the GABA<sub>B</sub> receptor systems. The pattern of expression of the CB<sub>1</sub> receptors strongly overlaps with that of the GABA<sub>B</sub> and the μ-opioid receptors in certain central nervous systems (CNS) regions. All three receptor systems are predominantly coupled to heterotrimeric G<sub>i/o</sub>-proteins. Several studies have revealed a functional interaction of the CB<sub>1</sub> receptors with the GABA<sub>B</sub> and the MORs at the level of G-proteins in certain regions of the CNS. Importantly, CB<sub>1</sub>, GABA<sub>B</sub> and MORs have been shown to display similar pharmacological effects, particularly on pain.

The first highly selective CB<sub>1</sub> receptor antagonist, SR141716 that was used in the clinics under the name Rimonabant to reduce obesity has been shown to exert a plethora of pharmacological effects in a number of pathological conditions. These effects are mainly attributed to its antagonistic properties at the CB<sub>1</sub> receptors, although there is increasing evidence that it may also behave as an inverse agonist. However, recent studies have revealed the existence of CB<sub>1</sub> receptor-independent actions of CB<sub>1</sub> inverse agonists. It has been proposed that the inhibitory effect of SR141716 on the basal receptor activity might occur either via a non-receptor-mediated effect or by binding to a site other than the agonist binding site on the CB<sub>1</sub> receptors, or by binding to GPCRs other than the CB<sub>1</sub> receptors, to which it binds with much lower affinity. Although there are data supporting these notions, the exact mechanism of inverse agonism by SR141716 has not been clarified yet.

The GABA<sub>B</sub> receptors are highly unusual among GPCRs in their requirement for heterodimerization between two subunits, the GABA<sub>B1</sub> and the GABA<sub>B2</sub> for functional expression. Immuno-electron microscopic studies have suggested that the GABA<sub>B2</sub> subunit may be absent, but electrophysiological data have shown the presence of functional GABA<sub>B</sub> autoreceptors in CCK-containing interneurons in rat hippocampus (*T. Freund, personal communication*). This observation raises the possibility that the GABA<sub>B1</sub> may function in association with additional interacting

partners, for example a yet unidentified GABA<sub>B</sub> receptor subunit, a distinct GPCR, or a chaperoning protein.

## **AIM OF THE STUDIES**

The present work consists of two distinct, but related studies about the promiscuity of the CB<sub>1</sub> receptor system. In the first part, our goal was to reveal possible interaction between the CB<sub>1</sub> and GABA<sub>B</sub> receptors. Previous literature data have raised the possibility that the well-known CB<sub>1</sub> receptor antagonist, SR141716 may have some non-CB<sub>1</sub> receptor mediated inverse agonist effects. Thereby, in the second part of our work, we have performed a detailed study on the promiscuous action of SR141716.

### **The current work focused on**

**1.** Investigating if there is functional interaction of the CB<sub>1</sub> and GABA<sub>B</sub> receptors at the G-protein level in rat hippocampus. Thereby, we have studied:

- if there are functional GABA<sub>B</sub> and cannabinoid CB<sub>1</sub> receptors in rat hippocampal membranes;
- whether CB<sub>1</sub> and GABA<sub>B</sub> receptors interact on G-protein signaling and if so what are the pharmacological consequences;

- what may be the mechanism? Cross-talk, hetero-oligomerization, or?

**2.** Assessing the inverse agonist effect of SR141716. The following questions have been investigated:

- is the inverse agonist effect mediated via the CB<sub>1</sub> receptors?  
Under what conditions?
- or does it occur via binding to GPCRs other than the CB<sub>1</sub> receptors, e.g. MOR?
- or is it a non-receptor-mediated effect?

Hence, we have used tissues containing distinct populations of receptors that **a**) contain both the CB<sub>1</sub> receptors and the MORs (wild-type, wt mouse cerebral cortex); **b**) lack the CB<sub>1</sub> receptors (CB<sub>1</sub> receptor knock-out, CB<sub>1</sub>-KO mouse cerebral cortex); **c**) lack both the CB<sub>1</sub> receptors and the MORs (parental Chinese hamster ovary, CHO cells); or **d**) contain a homogeneous population of over-expressed recombinant MORs (MOR-CHO cells), which were either untreated or made morphine-tolerant.

## METHODS

### Animals

Rats (Wistar male, 200-250 g, inbred in the BRC, Szeged, Hungary) and mice (CD1 male, 20-25 g) were handled in accordance with the European Communities Council Directives (86/609/EEC), and the Hungarian Act for the Protection of Animals in Research (XXVIII.tv. Section 32). CB<sub>1</sub>-KO mutant mice generated as described (Ledent et al., 1999, *Science* 283, 401-404) were provided by Dr. Freund, Institute of Experimental Medicine, Budapest, Hungary).

### Brain membrane preparation

Animals were decapitated, their brains removed, followed by dissection of hippocampus or cortex on ice. The tissues were washed with ice-cold buffer and their weight measured. They were homogenized in 30 volume (v/w) of ice-cold 50 mM Tris-HCl buffer (pH 7.4) with 5 strokes in a teflon-pestle Braun homogenizer at 1500 U/min. Homogenates were centrifuged at 20,000 x g for 25 min, the resulting pellets suspended in buffer and spun again. Pellets were taken up in the original volume of buffer and incubated for 30 min at 37 °C, followed by centrifugation at 20,000 x g for 25 min. The supernatants were carefully discarded, and the final pellets taken up in 5 volumes (v/w) of 50 mM Tris-HCl buffer (pH 7.4) containing 0.32 M sucrose. Appropriate membrane aliquots were stored at -80 °C for several weeks.

### Rat spinal cord membrane preparation

Rat spinal cords were dissected and stored at -80 °C for several weeks. They were thawed before use and homogenized in 10 volume (v/w) of ice-cold 50 mM Tris-HCl buffer (pH 7.4) with 5 strokes in a teflon-pestle Braun homogenizer at 700 U/min. Homogenates were centrifuged at 5,000 x g for 10 min. The supernatant was carefully decanted and stored on ice. The pellet was suspended in the original volume of buffer and spun as above. The combined supernatants of the two centrifugation steps were spun at 20,000 x g for 25 min. The resulting pellet was taken up in the original volume of buffer and incubated for 30 min at 37 °C, followed by

centrifugation at 20,000 x g for 25 min. The supernatant was carefully discarded. The final pellets were taken up in 20 volumes (v/w) of 50 mM Tris-HCl buffer (pH 7.4) and freshly used in the functional assay.

### **Cell culture and treatment**

CHO cells stably transfected with the MORs (MOR-CHO) were cultured as previously described (Szücs et al., 2004, *J Pharmacol Exp Ther* 310, 256-262). Briefly, the cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) high glucose with L-glutamine supplemented with 10% fetal calf serum. 1% penicillin/streptomycin and 3.6% geneticin.. Cells were grown at 37°C in a humidified atmosphere of 10% CO<sub>2</sub>, 90% air. One set of cells were treated with 100 ng/ml pertussis toxin (PTX) for the last 24 h in culture. At the end of PTX exposure, the cells were washed twice with ice-cold phosphate-buffered saline (PBS). The cells were harvested with PBS containing 1 mM EDTA. Cell suspension was spun at 2,500 rpm for 5 min, after which preparation of the cell membranes commenced.

### **Cell membrane preparation**

Freshly collected cell pellets were homogenized with a Wheaton teflon-glass homogenizer in 10 vols (v/w) of ice-cold homogenization buffer, pH 7.4, composed of 25 mM HEPES, 1 mM EDTA, 0.5 mg/l aprotinin, 1 mM benzamidine, 100 mg/l bacitracin, 3.2 mg/l leupeptin, 3.2 mg/l soybean trypsin inhibitor and 10% sucrose as reported earlier (Szücs et al., 2004, *J Pharmacol Exp Ther* 310, 256-262). Homogenates were spun at 1,000 x g for 10 min at 4 °C, and the supernatant was collected. Pellets were suspended in half of the original volumes of the homogenization buffer and centrifuged as above. Combined supernatants from the two low-speed centrifugations were spun at 20,000 x g for 30 min. The cell pellets were taken up in appropriate volumes of homogenization buffer. Aliquots were stored at -80 °C until use.

## **Protein determination**

The protein content of the membrane preparations was determined by the method of Bradford, BSA being used as a standard (Bradford, 1976, *Anal Biochem* 36, 708-719).

## **Ligand-stimulated [<sup>35</sup>S]GTPγS binding assay**

The assay was performed as described (Fabian et al., 2002) except that the concentration of GDP (3 μM) and NaCl (100 mM) was optimized for both CB<sub>1</sub> and GABA<sub>B</sub> agonists in rat hippocampus, cortex and spinal cord in preliminary experiments. The concentration of GDP was optimized at 3 and 30 μM for MOR-CHO cells and mouse cortex membranes, respectively. The highest concentrations of the solvents (0.1% ethanol or DMSO) tested in preliminary experiments had no effect on the basal activity in the assay. Briefly, crude membrane fractions (10 μg of protein) were incubated with 0.05 nM [<sup>35</sup>S]GTPγS and appropriate concentrations of ligands in TEM buffer (50 mM Tris-HCl, 1 mM EGTA and 3 mM MgCl<sub>2</sub>, pH 7.4) containing appropriate concentrations of GDP, 100 mM NaCl and 0.1% (w/v) BSA in a total volume of 1 ml for 60 min at 30 °C. Nonspecific binding was determined with 10 μM GTPγS and subtracted to yield specific binding values. Bound and free [<sup>35</sup>S]GTPγS were separated by vacuum-filtration through Whatman GF/F filters with a Brandel Cell Harvester . Filters were washed with 3 × 5 ml of ice-cold buffer, and radioactivity of the dried filters was detected in a toluene-based scintillation cocktail in a Wallac 1409 scintillation counter. .

## **Radioligand binding assay**

Heterologous displacement assays were performed with a constant concentration (1 nM) of [<sup>3</sup>H]DAMGO (spec. activity 36 Ci/mmol), 11 concentrations (10<sup>-10</sup>-10<sup>-5</sup> M) of unlabeled Win55,212-2 or SR141716 and MOR-CHO membrane suspension (10 μg protein) in 50 mM Tris-HCl pH 7.4 buffer containing 0.1% (w/v) BSA in a final volume of 1 ml. Nonspecific binding was defined as the radioactivity bound in the presence of 10 μM unlabeled naloxone, and was subtracted from the total binding to obtain the specific binding. The tubes were incubated at 25 °C for 1 h. The reaction was stopped by vacuum filtration through Whatman GF/C glass fiber

filters using a Brandel M24-R Cell Harvester. Filters were rapidly washed with 3 x 5 ml of ice-cold 50 mM Tris-HCl pH 7.4 buffer, air-dried and counted in a toluene-based scintillation cocktail in a Wallac 1409 scintillation counter. All assays were performed in duplicate and repeated at least three times.

### Data analysis

The dose-response curves in the ligand-stimulated [<sup>35</sup>S]GTP $\gamma$ S binding assay were drawn and analyzed with the GraphPad Prism 4.0 software using nonlinear regression and sigmoidal curve fitting to obtain potency ( $EC_{50}$ : the ligand concentration that elicits half-maximal effect) and efficacy ( $E_{max}$ : maximal effect) values. Basal activities were measured in the absence of receptor ligands and defined as 0% in each experiment unless otherwise indicated. All data are expressed as percentages of the basal [<sup>35</sup>S]GTP $\gamma$ S binding and are the means  $\pm$  S.E.M. of the result of at least three independent experiments performed in triplicate.  $IC_{50}$  (the concentration of ligand required to achieve 50% inhibition) values were obtained from the radioligand displacement curves using GraphPad Prism Software. All receptor binding data are expressed as percentage inhibition of specific binding and are the means  $\pm$  S.E.M. of the result of at least three independent experiments performed in duplicate. Statistical analysis was performed with GraphPad Prism, using ANOVA or Student's t-test analysis. Significance was defined at  $p < 0.05$  level.

## **SUMMARY OF THE RESULTS**

**The main results are the following:**

- 1) The GABA<sub>B</sub> receptor antagonist, phaclofen at low doses (1 and 10 nM) significantly attenuated G-protein signaling of the CB<sub>1</sub> agonist Win55,212-2 in rat hippocampal membranes. High concentrations of phaclofen had no effect, ruling out the possibility of direct action of the GABA<sub>B</sub> receptor antagonist on the CB<sub>1</sub> receptors. It was also shown that the interaction is sterospecific suggesting receptor-mediated action.
- 2) The specific CB<sub>1</sub> antagonist AM251 at a low dose (1 nM) also inhibited the efficacy of G-protein signaling of the GABA<sub>B</sub> receptor agonist SKF97541 in rat hippocampal membranes. It is speculated that the interaction might occur via an allosteric interaction between a subset of GABA<sub>B</sub> and CB<sub>1</sub> receptors in rat hippocampal membranes.
- 3) Cross-talk of the CB<sub>1</sub> and GABA<sub>B</sub> receptor systems was not detected in either spinal cord or cerebral cortex membranes. These results show that the interaction between CB<sub>1</sub> and GABA<sub>B</sub> receptors is tissue specific.
- 4) SR141716 (10 μM) significantly decreased the basal [<sup>35</sup>S]GTPγS binding in membranes of wild-type and CB<sub>1</sub> receptor knock-out mouse cortex, parental Chinese hamster ovary (CHO) cells and CHO cells stably transfected with MOR,

MOR-CHO. Accordingly, we conclude that the inverse agonism of SR141716 is CB<sub>1</sub> receptor-independent.

- 5) It was demonstrated that SR141716 directly bind to MORs, albeit with low affinity.
- 6) After pertussis toxin (PTX) treatment that uncouples MORs from G<sub>i</sub>/G<sub>o</sub> proteins, the inverse agonism of SR141716 was abolished, and DAMGO alone displayed weak, naloxone-insensitive stimulation. Notably, the combination of DAMGO and SR141716 (10 μM each) resulted in a 169 ± 22% stimulation of the basal activity that was completely inhibited by the prototypic opioid antagonist naloxone in MOR-CHO membranes, thereby revealing novel, non-PTX mediated G-protein signaling of MOR.
- 7) Chronic morphine treatment caused desensitization of the novel G-protein signaling induced by co-addition of DAMGO and SR141716 to MOR-CHO.

**Consequently**, these data revealed **reciprocal inhibition of G-protein signaling induced by CB<sub>1</sub> and GABA<sub>B</sub> receptors in rat hippocampus**. It is intriguing that the cross-talk between CB<sub>1</sub> and GABA<sub>B</sub> receptors might be involved in balance tuning the endocannabinoid and GABAergic signaling in hippocampus. In addition, CB<sub>1</sub> receptor-independent actions of SR141716 occurred on G-protein signaling. Its co-application with the μ-opioid agonist DAMGO unmasked novel, pertussis toxin-insensitive opioid

signaling in MOR-CHO cells. We concluded that **SR141716 exerts multifaceted effects on G-protein signaling**. It is anticipated that it may also affect the signaling of other GPCRs. **The multifaceted actions of the SR141716 should be taken into account when applied in high doses in the clinics.**

Receptor and ligand interactions at new levels reveal previously not recognized complexity of receptor functioning that may provide important data for both basic and pharmaceutical research fields.

## LIST OF PUBLICATIONS

### Publications related to the thesis:

1. **Cinar R.**, Freund TF, Katona I, Mackie K, and Szucs M (2008) Reciprocal inhibition of G-protein signaling is induced by CB<sub>1</sub> cannabinoid and GABA<sub>B</sub> receptor interactions in rat hippocampal membranes. *Neurochem Int* 52: 1402–1409.  
(IF:3.228)
2. **Cinar R.** and Szücs M (2009) CB<sub>1</sub> receptor-independent actions of SR141716 on G-protein signaling; co-application with the  $\mu$ -opioid agonist DAMGO unmasks novel, pertussis toxin-insensitive opioid signaling in MOR-CHO cells. *J Pharmacol Exp Ther* 330 (2) 567-574.  
(IF:4.309)

### Abstracts Published in Referred Journal:

1. Szucs M., and **Cinar R.** CB<sub>1</sub> receptor-independent effects of the CB<sub>1</sub> receptor antagonist/inverse agonist rimonabant (SR141716). (2009). 12th Meeting of the Hungarian Neuroscience Society, Budapest, Hungary published in *Frontiers in Systems Neuroscience* doi: 10.3389/conf.neuro.01.2009.04.242
2. **Cinar R.**, Freund T.F., Katona I., Mackie K., Szucs M. Cross-talk between cannabinoid CB<sub>1</sub> and GABA<sub>B</sub> receptors in rat brain hippocampus. (2008). Szeged University Biology Ph.D. School Seminars, Szeged, Hungary published in *Acta Biologica Szegediensis* 52(2) p.335.
3. **Cinar R.**, Mackie K., Freund T.F., Szucs M. Searching for possible interactions between CB<sub>1</sub> and GABA<sub>B</sub> receptors in rat brain hippocampal membranes (2006) 31st FEBS

Congress, Istanbul, Turkey. PP-54; published in *The FEBS Journal*, 273 (Suppl 1.) p.91.

### Abstracts Published in Journal:

- 1- **Cinar R.**, Katona I., Mackie K., Freund T.F., Szucs M. Reciprocal modulation of G-protein signaling between cannabinoid CB<sub>1</sub> and GABA<sub>B</sub> receptors in rat brain hippocampus (**2008**). IBRO international workshop 2008, Debrecen, Hungary. P97; published in *Clinical Neuroscience*, 61 (S1) p.18-19.
- 2- Kekesi O., Birkas E., **Cinar R.**, Szucs M. Molecular changes accompanying morphine withdrawal in rat brain. (**2008**) IBRO international workshop 2008, Debrecen, Hungary. P72; published in *Clinical Neuroscience*, 61 (S1) p.38
- 3- **R. Cinar**, I. Katona, T.F. Freund, K. Mackie, M. Szucs. Cannabinoid CB<sub>1</sub> and GABA<sub>B</sub> receptor interactions:reciprocal inhibition of G-protein signalling in rat brain hippocampus. (**2008**).MBKE, Szeged, Hungary. P-64; published in *Biokémia*, XXXII (3.) p.70
- 4- Kekesi O., Birkas E., **Cinar R.**, Lidia B., Karoly G., Szucs M. Morfin dependencia és megvonás hatása a μ-opioid receptorok kötési paramétereire patkány agyban. (**2008**). MBKE, Szeged, Hungary. P-63; published in *Biokémia*, XXXII (3.) p.70
- 5- **Cinar R.**, Mackie K., Freund T.F., Szucs M. Interaction between cannabinoid and GABA<sub>B</sub> receptors in rat brain hippocampus. (**2007**) 11<sup>th</sup> Annual meeting of the Hungarian Neuroscience society, Szeged, Hungary. P181; published in *Clinical Neuroscience*, 60 (S1) p.13
- 6- **Cinar R.**, Mackie K., Freund T.F., Szucs M. Interplay between CB<sub>1</sub> and GABA<sub>B</sub> receptors in rat brain

hippocampal membranes. (2006) MBKE , Pécs, Hungary.  
E7-10; published in *Biokémia*, XXX (3.) p.62

### Oral Presentations:

1. **Cinar R.**, Bir Tango Hikayesi: Rezeptör Seçiciliği ve Kompleksliği. Invited Seminar (2009), Faculty of Pharmacy, Ege University, Izmir, Turkey
2. **Cinar R.**, Receptor selectivity and promiscuity. (2009) Biochemistry Seminars, Biological Research Center, Szeged, Hungary
3. **Cinar R.**, Freund T.F., Katona I., Mackie K., Szucs M. Cross-talk between cannabinoid CB<sub>1</sub> and GABA<sub>B</sub> receptors in rat brain hippocampus. (2008) Szeged University Biology Ph.D. School Seminars, Szeged, Hungary
4. **Cinar R.**, Freund T.F., Katona I., Mackie K., Szucs M. Receptor-receptor interactions; implications in modulating receptor function. (2007) Peptid Chemistry Conference, Balatonszemes, Hungary
5. **Cinar R.**, Mackie K, Freund T.F., Szucs M., It takes two to tango: interaction between cannabinoid and GABA<sub>B</sub> receptors in rat brain hippocampus. (2006) Straub-Days, Biological Research Center, Szeged, Hungary
6. **Cinar R.**, Mackie K, Freund T.F., Szucs M. Interplay between CB<sub>1</sub> and GABA<sub>B</sub> receptors in rat brain hippocampal membranes. (2006) MBKE, Pécs, Hungary  
E7-10
7. **Cinar R.**, Searching for possible interactions between CB<sub>1</sub> and GABA<sub>B</sub> receptors in hippocampus. (2006) Biochemistry Seminars, Biological Research Center, Szeged, Hungary

8. **Cinar R.**, Studies on homo- and hetero-oligomerization of distinct g-protein coupled receptors. (2005) in proceedings of the closing seminar of the International Training Course 2004/2005 Biological Research Center, Szeged, Hungary.

### Poster Presentations:

1. **Cinar R.** and Szucs M. From a single target to promiscuity; CB<sub>1</sub> receptor-independent actions of rimonabant (SR141716) on G-protein signalling. (2009) 22nd European College of Neuropsychopharmacology (ECNP) Congress, Istanbul, Turkey– P.1.c.036
2. **Cinar R.** and Szucs M. CB<sub>1</sub> receptor-independent effects of the CB<sub>1</sub> receptor antagonist/inverse agonist rimonabant (SR141716). (2009) 12<sup>th</sup> annual meeting of the Hungarian Neuroscience Society, Budapest, Hungary – P080 p.35
3. **Cinar R.**, Katona I., Mackie K., Freund T.F., Szucs M. Cannabinoid CB<sub>1</sub> and GABA<sub>B</sub> receptor interactions: reciprocal inhibition of G-protein signaling in rat brain hippocampus. (2008) MBKE, Szeged, Hungary P.64
4. Kekesi O., Birkas E., **Cinar R.**, Lidia B., Karoly G., Szucs M. Morfin dependencia és megvonás hatása a μ-opioid receptorok kötési paramétereire patkány agyban. (2008) MBKE, Szeged, Hungary P.63
5. **Cinar R.**, Katona I., Mackie K., Freund T.F., Szucs M. Reciprocal inhibition of G-protein signaling between cannabinoid CB<sub>1</sub> and GABA<sub>B</sub> receptors in rat brain hippocampal membranes. (2008) 38. Membrane-Transport Conference, Sumeg, Hungary P.69.
6. Kekesi O., Birkas E., **Cinar R.**, Szucs M. Molecular changes associated with morphine withdrawal in rat brain. (2008) 38. Membrane-Transport Conference, Sumeg, Hungary P.32

7. **Cinar R.**, Katona I., Mackie K., Freund T.F., Szucs M. Cross-inhibition of G-protein signaling with cannabinoid CB<sub>1</sub> and GABA<sub>B</sub> receptors in rat brain hippocampus. (2008) EOC-ENC Joint Meeting, Ferrara, Italy P.I.3.
8. **Cinar R.**, Katona I., Mackie K., Freund T.F., Szucs M. Reciprocal modulation of G-protein signaling between cannabinoid CB<sub>1</sub> and GABA<sub>B</sub> receptors in rat brain hippocampus. (2008) IBRO international workshop, Debrecen, Hungary P97
9. Kekesi O., Birkas E., **Cinar R.**, Szucs M. Molecular changes accompanying morphine withdrawal in rat brain. (2008) IBRO international workshop, Debrecen, Hungary P72
10. **Cinar R.**, Katona I., Mackie K., Freund T.F., Szucs M. Reciprocal modulation between G-protein signaling of cannabinoid CB<sub>1</sub> and GABA<sub>B</sub> receptors in rat brain hippocampus. (2007) 37<sup>th</sup> annual meeting of the society for Neuroscience, San Diego, California, USA 578.3/L25
11. **Cinar R.**, Katona I., Mackie K., Freund T.F., Szucs M. Reciprocal modulation between G-protein signaling of cannabinoid CB<sub>1</sub> and GABA<sub>B</sub> receptors in rat brain hippocampus. (2007) NIDA Mini Convention: Frontiers in Addiction Research, San Diego, California, USA- P.20
12. **Cinar R.**, Katona I., Mackie K., Freund T.F., Szucs M. Reciprocal modulation between G-protein signaling of cannabinoid CB<sub>1</sub> and GABA<sub>B</sub> receptors in rat brain hippocampus. (2007) 17<sup>th</sup> Neuropharmacology conference: Cannabinoid signaling in the nervous system, San Diego, California, USA P1.1.01
13. **Cinar R.**, Mackie K., Freund T.F., Szucs M. Interaction between cannabinoid and GABA<sub>B</sub> receptors in rat brain

hippocampus. (2007) 11<sup>th</sup> annual meeting of the Hungarian Neuroscience Society, Szeged, Hungary - P181 p.41

14. **Cinar R.**, Mackie K., Freund T.F., Szucs M. Searching for possible interactions between CB<sub>1</sub> and GABA<sub>B</sub> receptors in rat brain hippocampal membranes (2006) 1st ITC Alumni Meeting “Modern Trends in Biological Sciences: Seeking for an integrating approach”, Biological Research Center, Szeged, Hungary A-5 p.41
15. **Cinar R.**, Mackie K., Freund T.F., Szucs M. Searching for possible interactions between CB<sub>1</sub> and GABA<sub>B</sub> receptors in rat brain hippocampal membranes (2006) 31<sup>st</sup> FEBS Congress, Istanbul, Turkey PP-54

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