

**11ª Reunión Anual**  
**Sociedad Española de Investigación sobre**  
**Cannabinoides**  
**Pontevedra, 25 a 27 de noviembre de 2010**

**Programa científico de la Reunión**

**Jueves, 25 de noviembre**

- 12:30-14:00**      **Entrega de documentación** (Centro Social Caixanova de Pontevedra, c/ Augusto González Besada, 2)
- 14:00-16:00**      **Comida**
- 16:00-16:30**      **Inauguración**
- Miguel Anxo Fernández Lores, Excmo. Alcalde de Pontevedra
  - Teresa Pedrosa Silva, Vicepresidenta de la Deputación de Pontevedra
  - Ruth Pazos, Presidenta del Comité Organizador
  - Javier Fernández Ruiz, Presidente de la SEIC
- 16:30-17:30**      **Conferencia Inaugural** (presentada por Manuel Guzmán)  
Christopher Fowler, Universidad de Umea, Suecia:  
"The role of the endocannabinoid system in prostate cancer"
- 17:30-19:00**      **Mesa Redonda:** "Sativex® and other cannabinoid-based medicines" (moderadores: Javier Fernández Ruiz y Manuel Guzmán)
- Stephen Wright, GW Pharmaceuticals, Reino Unido
  - Eduardo Muñoz, VivaCell Biotechnology, España
  - Christopher Fowler, Universidad de Umea, Suecia
  - José Martínez Orgado, Hospital Universitario Puerta de Hierro Majadahonda, España
- Proyección** del vídeo conmemorativo del 80 aniversario de Raphael Mechoulam
- 19:00-19:30**      **Café**
- 19:30-20:00**      **Presentación del Premio a la Mejor Publicación 2010**  
(presentado por Emilio Fernández Espejo)

20:00 **Visita guiada por la ciudad de Pontevedra y recepción ofrecida por el Excmo. Ayuntamiento de Pontevedra en el Ayuntamiento**

**Viernes, 26 de noviembre**

09:00 **Presentación 1ª Sesión de comunicaciones orales**  
(moderadores: Pedro Grandes y Nadine Jagerovic)

09:15-11:45 **1ª Sesión de comunicaciones orales: "Biología y farmacología del sistema endocannabinoide"**

09:15 O-1.1  
CB<sub>1</sub> CANNABINOID RECEPTOR DISTRIBUTION IN RAT CEREBELLUM DURING EARLY POSTNATAL DEVELOPMENT. I. Buceta, N. Puente, L. Reguero, J.L. Mendizabal-Zubiaga, M.J. Canduela, P. Grandes, and I. Elezgarai.

09:30 O-1.2  
INTRINSIC MECHANISMS OF THE PRODUCTION OF 2-AG AND AEA MEDIATING DIFFERENT FORMS OF SYNAPTIC PLASTICITY IN A SINGLE NEURON. N. Puente, I. Elezgarai, L. Reguero, I. Buceta, J.L. Mendizabal-Zubiaga, M.J. Canduela, P. Grandes, and O.J. Manzoni.

09:45 O-1.3  
ENDOCANNABINOIDS POTENTIATE SYNAPTIC TRANSMISSION THROUGH ASTROCYTE STIMULATION. M. Navarrete, and A. Araque.

10:00 O-1.4  
CB<sub>1</sub> CANNABINOID RECEPTORS REGULATE PYRAMIDAL NEURON SPECIFICATION. J. Díaz, T. Aguado, J. Palazuelos, C.S. Wu, B. Lutz, H.C. Lu, M. Guzmán, and I. Galve-Roperh.

10:15 O-1.5  
EFFECTS OF NEONATAL MATERNAL DEPRIVATION AND/OR A HIGH FAT DIET ON HYPOTHALAMIC AND HIPPOCAMPAL ENDOCANNABINOID LEVELS IN MALE AND FEMALE RATS. V. Mela, G.K. Ford, M. Valerol, D. Kerr, E.M. Marco, M. Roche, D.P. Finn, and M.P. Viveros.

10:30 O-1.6  
STUDY OF THE POLYGENIC INFLUENCE ON THE CANNABINOID TETRAD TESTS USING CONSOMIC MICE STRAINS. M. Tuda-Arizcun, T. Koide, S. Toshihiko, V. Echeverry-Alzate, K.M. Bühler, B. Lutz, and J.A. López-Moreno.

10:45 O-1.7  
DIFFERENTIAL ROLE OF ENDOCANNABINOIDS IN MEMORY, NOCICEPTION AND ANXIETY-LIKE RESPONSES. A. Busquets-

García, E. Puighermanal, A. Pastor, R. de la Torre, R. Maldonado, and Andrés Ozaita.

- 11:00 O-1.8  
SPATIAL MEMORY IMPAIRMENT IN DEVELOPMENTAL HYPOTHYROID RATS: IMPLICATION OF THE ENDOCANNABINOID SYSTEM. E. Giné, V. Echeverry-Alzate, J.A. López-Moreno, M. Tuda Arizcun, K.M. Buhler, A. López-Jiménez, A. Pérez-Castillo, and A. Santos.
- 11:15 O-1.9  
CHRONIC DELTA9-TETRAHYDROCANNABINOL AFFECTS CEREBELLAR FUNCTION THROUGH THE ACTIVATION OF CEREBELLAR MICROGLIA. L. Cutando, E. Puighermanal, A. Busquets-García, J.M. Delgado-García, A. Gruart, R. Maldonado, and A. Ozaita.
- 11:30 O-1.10  
CANNABINOID RECEPTOR I (CB<sub>1</sub>) RECEPTORS ARE PRESENT IN PANCREATIC POLYPEPTIDE AND DELTA CELL SUBPOPULATIONS WITHIN MOUSE PANCREATIC ISLETS. F. Aragón, M. Bombardó, A. Ozaita, R. Maldonado, and B. Rubí.
- 11:45-13:00** **Café y sesión de pósters**
- 13:00** **Presentación 2ª Sesión de comunicaciones orales**  
(moderadores: Inés Díaz-Laviada y Juan Suárez)
- 13:15-14:00** **2ª Sesión de comunicaciones orales: "Cannabinoides y cáncer"**
- 13:15 O-2.1  
ROLE OF THE CB<sub>2</sub> CANNABINOID RECEPTOR IN ERBB2-DRIVEN BREAST CANCER PROGRESSION. M.M. Caffarel, E. Pérez-Gómez, C. Andradás, J.M. Flores, M. Guzmán, and C. Sánchez.
- 13:30 O-2.2  
CANNABINOID-BASED COMBINED THERAPIES AS A NOVEL STRATEGY TO FIGHT GLIOMAS? S. Torres, M. Lorente, F. Rodríguez, S. Hernández-Tiedra, M. Salazar, E. García-Taboada, M. Guzmán, and G. Velasco.
- 13:45 O-2.3  
BASALIOMA TREATMENT WITH INTRATUMORAL INJECTIONS OF A CANNABIS-OIL EXTRACTION: RELATED CASE. J. Pedraza-Valiente.
- 14:00-16:00** **Comida**

- 16:00** **Presentación 3ª Sesión de comunicaciones orales**  
(moderadores: Pilar Sánchez-Blázquez y Rosa Tolón)
- 16:15-17:45** **3ª Sesión de comunicaciones orales: "Cannabinoides como drogas de abuso"**
- 16:15 O-3.1  
GABA/GLUTAMATE IMBALANCE IN THE HIPPOCAMPUS OF ADULT FEMALE RATS EXPOSED TO CANNABINOIDS DURING ADOLESCENCE. A. Higuera-Matas, M. Miguéns, S.M. Coria, A. Assís, G.L. Montoya, N. del Olmo, and E. Ambrosio.
- 16:30 O-3.2  
A STUDY ON WIN 55,512-2 SELF-ADMINISTRATION BEHAVIOUR IN LEWIS AND FISCHER 344 RATS. S.M. Coria, M. Miguéns, A. Assís, G.L. Montoya, N. del Olmo, C. García-Lecumberri, A. Higuera-Matas, and E. Ambrosio.
- 16:45 O-3.3  
COCAINE SELF-ADMINISTRATION DIFFERENTIALLY MODULATES ENDOGENOUS CANNABINOID SYSTEM PROTEINS IN THE RAT HIPPOCAMPUS OF FISHER AND LEWIS RATS. P. Rivera, M. Miguéns, S. Morales, A. Higuera-Matas, F.J. Bermúdez-Silva, F. Rodríguez de Fonseca, J. Suárez, and E. Ambrosio.
- 17:00 O-3.4  
MATERNAL DEPRIVATION EFFECTS ON MDMA INDUCED CONDITIONED PLACE PREFERENCE IN ADOLESCENT RATS AND SEX- DEPENDENT CHANGES IN HIPPOCAMPAL CB<sub>1</sub> RECEPTOR. A. Llorente-Berzal, C. Manzanedo, J. Miñarro, and M.P. Viveros.
- 17:15 O-3.5  
EFFECTS OF CANNABIS CONSUMPTION IN SUBCLINICAL NEGATIVE PSYCHOTIC EXPERIENCES. L. Hernández, J.L. Rubio, and M. Ruiz-Veguilla.
- 17:30 O-3.6  
HUMAN SINGLE NUCLEOTIDE POLYMORPHISMS (SNP´s) FROM THE CANNABINOID AND CATECHOLAMINERGIC SYSTEM ARE ASSOCIATED TO DRUG CONSUMPTION AND BASIC PSYCHOLOGICAL PROCESSES. K.M. Bühler, E. Huertas, V. Echerverry-Alzate, M. Tuda-Arizcun, and J.A. López-Moreno.
- 17:45-19:00** **Café y sesión de pósters**
- 19:00-20:30** **Asamblea de la SEIC**
- Cena (libre)

## Sábado, 27 de noviembre

- 9:00**                    **Presentación 4ª Sesión de comunicaciones orales**  
(moderadores: Moisés García-Arencibia y José Martínez-Orgado)
- 9:15-12:00**           **4ª Sesión de comunicaciones orales: "Cannabinoides y neuroprotección"**
- 09:15                    O-4.1  
WIN 55,212-2 ATENUATES BRAIN DAMAGE AFTER PERINATAL HYPOXIC-ISCHEMIC INJURY. D. Alonso-Alconada, A. Álvarez, F. J. Alvarez, S. Girelli, I. Lara-Celador, O. Arteaga, F. Goñi-de-Cerio, J. Lacalle, W. Balduini, J. Martínez-Orgado, and E. Hilario.
- 09:30                    O-4.2  
STUDY OF THE ADMINISTRATION OF 2-ARACHIDONYL GLYCEROL AND ANANDAMIDE ON MITOCHONDRIAL STATE, GLIAL RESPONSE AND CELL DEATH IN A HYPOXIC-ISCHEMIC RAT MODEL. I. Lara-Celador, L. Castro, A. Álvarez, J. Lacalle, D. Alonso-Alconada, F.J. Álvarez, J. Martínez-Orgado, L. Álvarez-Granda, and E.Hilario.
- 09:45                    O-4.3  
HISTOLOGICAL NEUROPROTECTION OF CANNABIDIOL AS FUNCTION OF TIME IN HIPOXIC-ISCHEMIC ENCEPHALOPATHY. H. Lafuente, M.R. Pazos, J. Martínez-Orgado, M.C. Rey-Santano, V. Mielgo, X. Murgia-Esteve, I. Valls, A. Soler, and F.J. Álvarez.
- 10:00                    O-4.4  
CEREBRAL AND EXTRACEREBRAL EFFECTS OF CBD IN PIGLETS AFTER HYPOXIA-ISCHEMIA ARE MEDIATED BY CB<sub>2</sub> RECEPTORS. M.R. Pazos, L. Arruza, A. Gómez, M. Santos, F. Tendillo, H. Lafuente, E. Hilario, F.J. Álvarez, and J. Martínez-Orgado.
- 10:15                    O-4.5  
CANNABIDIOL REDUCES DISTANT LUNG INJURY SECONDARY TO HYPOXIC-ISCHEMIC BRAIN DAMAGE IN PIGLETS. L. Arruza, M.R. Pazos, A. Gómez, M. Santos, F. Tendillo, H. Lafuente, E. Hilario, F.J. Álvarez, and J. Martínez-Orgado.
- 10:30                    O-4.6  
CANNABINOIDS AMELIORATE DISEASE PROGRESSION IN A MODEL OF MULTIPLE SCLEROSIS IN MICE, ACTING THROUGH ANTI-INFLAMMATORY AND ANTI-GLUTAMATERGIC

MECHANISMS. M. Moreno-Martet, A. Cabranes (*in memoriam*), J.A. Ramos, J. Fernández-Ruiz, and E. de Lago.

10:45 O-4.7  
PROTECTIVE ROLE OF CANNABINOIDS IN AN EXPERIMENTAL MODEL OF ALZHEIMER'S DISEASE. E. Aso, E. Palomer, S. Juvés, R. Maldonado, F.J. Muñoz, and I. Ferrer.

11:00 O-4.8  
PROLONGED ORAL CANNABINOID ADMINISTRATION PREVENTS NEUROINFLAMMATION AND IMPROVES COGNITIVE DEFICITS IN A TRANSGENIC MODEL OF ALZHEIMER'S DISEASE. A.M. Martín-Moreno, B. Brera, N. Innamorato, A. Cuadrado, and M.L. de Ceballos.

11:15 O-4.9  
CHANGES IN SPECIFIC COMPONENTS OF THE ENDOCANNABINOID SYSTEM IN SPINOCEREBELLAR ATAXIAS. C. Rodríguez-Cueto, F. Espejo Porras, J. Fernández-Ruiz, M. Hernández-Gálvez, and M. Gómez-Ruiz.

11:45 O-4.10  
OLEYLETHANOLAMIDE IS A POWERFUL NEUROPROTECTANT IN DIFFERENT ANIMAL MODELS OF PARKINSONISM. R. González-Aparicio, J.A. Flores-Cordero, and E. Fernández-Espejo.

**12:15-13:30 Entrega de Premios a las Mejores Comunicaciones. Clausura y aperitivo.**

## SESIÓN DE PÓSTERS

### P.1

MODULATION OF BRAIN ENDOCANNABINOID SYSTEM DURING PRIMARY DEMYELINATION AND REMYELINATION. A. Bernal-Chico, C. Matute, and S. Mato.

### P.2

HYPOCRETINS ARE INVOLVED IN THE REINFORCING PROPERTIES OF THE SYNTHETIC CANNABINOID WIN 55,212-2. A. Flores, R. Maldonado, and F. Berrendero.

### P.3

LOSS OF STRIATAL CB1 CANNABINOID RECEPTORS IS A KEY PATHOGENIC FACTOR IN HUNTINGTON'S DISEASE. C. Blázquez, A. Chiarlone, O. Sagredo, T. Aguado, M.R. Pazos, E. Resel, J. Palazuelos, B. Julien, M. Salazar, C. Börner, C. Benito, C. Carrasco, M. Diez-Zaera, P. Paoletti, M. Díaz-Hernández, J. J. Lucas, J. G. de Yébenes, G. Marsicano, K. Monory, B. Lutz, J. Romero, J. Alberch, E. Pérez-Navarro, S. Ginés, J. Kraus, J. Fernández-Ruiz, I. Galve-Roperh, and M. Guzmán.

### P.4

CB<sub>1</sub> CANNABINOID RECEPTOR IN THE DEVELOPMENT AND EXPRESSION OF OSTEOARTHRITIS. C. La Porta, S.A. Bura, and R. Maldonado.

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AUTOPHAGY DURING NEUROENDOCRINE DIFFERENTIATION OF LNCaP CELLS. ROLE OF CANNABINOIDS. M.C. Morell, D. Vara, S. Pérez-Díaz, I. Díaz-Laviada, and N. Rodríguez-Henche.

### P.6

THE PUTATIVE CANNABINOID RECEPTOR GPR55 PROMOTES CANCER CELL PROLIFERATION VIA ERK. C. Andradas, M.M. Caffarel, E. Pérez-Gómez, M. Salazar, M. Lorente, G. Velasco, M. Guzmán, and C. Sánchez.

### P.7

SIMULTANEOUS SYMPTOM-RELIEVING AND NEUROPROTECTIVE TREATMENT WITH  $\Delta^9$ -THCV IN ANIMAL MODELS OF PARKINSON'S DISEASE. C. García, C. Palomo, M. García-Arencibia, J.A. Ramos, R.G. Pertwee, and J. Fernández-Ruiz.

### P.8

THE ACTIVATION OF THE ENDOCANNABINOID SYSTEM REDUCES THE TAT INDUCED-EXPRESSION OF PRO-INFLAMMATORY PARAMETERS IN GLIAL CELLS. C. Benito, A.I. Castillo, L. Ruiz-Valdepeñas, M. Moreno, J. Romero, and R.M. Tolón.

### P.9

ROLE OF mTOR IN THE PHARMACOLOGICAL EFFECTS OF THC. E. Puighermanal, A. Busquets-García, M. Gomis, R. Maldonado, and A. Ozaita.

**P.10**

COMPARATIVE STUDY OF DIFFERENT VEHICLES ON THE CANNABINOID TETRAD IN MICE. E. Sánchez, C. Goicoechea, and M.I. Martín.

**P.11**

HYDROSOLUBLE CANNABINOID COMPOUNDS: SYNTHESIS, BIOLOGICAL EVALUATION AND METABOLISM STUDIES. L. Moreno-Capellán, H. Guillén, L. Hernández-Folgado, R. Girón, T. Herraiz, M.I. Martín, M. Gómez, M. Gómez-Cañas, J. Fernández-Ruiz, P. Goya, and N. Jagerovic.

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THE INVERSE AGONIST EFFECT OF RIMONABANT IN HUMAN POSTMORTEM BRAIN IS MEDIATED BY  $G_{\alpha 13}/G_{\alpha o}$  PROTEINS. R. Diez-Alarcia, A.M. Erdozain, J.J. Meana, and L.F. Callado.

**P.13**

THE SPECIFIC  $CB_2$  AGONIST, HU-910, DOES NOT MODIFY DISEASE PROGRESSION IN AN ANIMAL MODEL OF ALZHEIMER'S DISEASE. L. Ruiz-Valdepeñas, C. Benito, M. Moreno, C. Vázquez, R.M. Tolón, and J. Romero.

**P.14**

PRECISE SUBCELLULAR LOCALIZATION OF THE  $CB_1$  RECEPTOR IN HIPPOCAMPAL GLIA. M.J. Canduela, N. Puente, I. Buceta, L. Reguero, J.L. Mendizabal-Zubiaga, K. Mackie, G. Marsicano, and P. Grandes.

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CANNABIDIOL PROTECTS OLIGODENDROCYTE PROGENITORS BY DIRECT AND INDIRECT MECHANISMS: IMMUNOMODULATORY ACTIONS IN A VIRAL MODEL OF MULTIPLE SCLEROSIS. M. Mecha, P. Íñigo, M. Hernangómez, L. Mestre, K. Goble, R. Mechoulam, and C. Guaza.

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NON- PSYCHOACTIVE CANNABINOIDS ATTENUATE MCP-1 EXPRESSION: IMPLICATIONS IN MULTIPLE SCLEROSIS. P.M. Íñigo, L. Mestre, M. Mecha, M. Hernangómez, and C. Guaza.

**P.17**

LONG TERM BENEFICIAL EFFECT OF CANNABIDIOL ON NEUROLOGICAL FUNCTION AFTER NEWBORN HYPOXIA-ISCHEMIA. M.R. Pazos, M. Santos, F. Tendillo, A. Gómez, L. Arruza, H. Lafuente, E. Hilario, F.J. Álvarez, and J. Martínez-Orgado.

**P.18**

THE LOSS OF HINT1 REVEALS THE NMDAR-SENSITIVE COMPONENT OF CANABINOID INDUCED ANALGESIA. P. Sánchez-Blázquez, A. Vicente-Sánchez, M. Rodríguez-Muñoz, E. Berrocoso, and J. Garzón.

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URB602, A MONOACYLGLYCEROL LIPASE INHIBITOR, PROTECTS FROM THE LONG-TERM CONSEQUENCES OF A NEONATAL HYPOXIC-ISCHEMIC BRAIN

INJURY IN RATS. S. Girelli, S. Carloni, A. Duranti, A. Tontini, D. Piomelli, D. Alonso-Alconada, E. Hilario, and W. Balduini.

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NEUROPROTECTIVE EFFECTS OF PHYTOCANNABINOID-BASED MEDICINES IN A RAT MODEL OF HUNTINGTON'S DISEASE. O. Sagredo, M.R. Pazos, S. Valdeolivas, V. Satta, R.G. Pertwee, and J. Fernández-Ruiz.

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REGULATION OF SOMATODENDRITIC 5-HT<sub>1A</sub> RECEPTORS BY URB597 ALONE OR IN COMBINATION WITH THE ANTIDEPRESSANT FLUOXETINE IN MICE. B.Treceño, E. Castro, A. Martín, and A. Pazos.

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EXPRESSION OF THE mRNAs CODING THE CANNABINOID RECEPTORS 1 AND 2 IN THE PALLIDAL COMPLEX OF MACACA FASCICULARIS. R. Franco, A.J. Rico, P. Barroso-Chinea, L. Callén, E. Roda, V. Gómez-Bautista, N. Luquin, S. Sierra, C. Lluís, J.L. Labandeira-García, and J.L. Lanciego.

## THE ROLE OF THE ENDOCANNABINOID SYSTEM IN PROSTATE CANCER

Christopher J. Fowler

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It is now well established that cannabinoids can affect the viability of cells from a number of different cancer types. *In vitro* studies can distinguish four main groups of action:

- CB receptor-mediated effects on cell viability seen at nanomolar cannabinoid concentrations
- CB receptor-mediated effects on cell viability seen at micromolar cannabinoid concentrations
- Effects of cannabinoids, usually at micromolar concentrations, upon cell viability that are not mediated by CB receptors
- Effects of CB receptor antagonists upon cell viability

*In vivo* studies have mainly used xenograft models, and data is needed not only in genetic models (such as reported for breast cancer by Caffarel *et al.*, *Mol Cancer* 9 [2010] 196) but also in combination studies with the current treatment strategies for the cancer in question.

The findings in the literature a) that the prostate expresses CB<sub>1</sub> receptors (Ruiz-Llorente *et al.*, *Prostate* 54 [2003] 95-102) and b) that modulation of endocannabinoid synthesis and degradation affect the *in vitro* invasivity of prostate cancer cell lines (Nithipatikom *et al.*, *Cancer Res* 64 [2004] 8826-30; Endsley *et al.*, *Int Cancer* 123 [2008] 1318-26) led us to investigate the endocannabinoid system in well characterised tissue microarrays from patients diagnosed with the disease at transurethral resection therapy for voiding problems. The studies can be summarised as follows:

- A high expression of CB<sub>1</sub> receptors measured in the tumour tissue biopsies at diagnosis is associated with a poor rate of disease-specific survival. This prognostic effect is additive to that provided by the Gleason score. No such association was seen for CB<sub>1</sub> receptors in non-malignant tissue (Chung *et al.*, *Eur J Cancer* 45 [2009] 174-82)
- A high expression of FAAH is associated both with an increased disease severity at diagnosis and with disease outcome. However, this effect is modulated by the level of expression of CB<sub>1</sub> receptors: a high CB<sub>1</sub> receptor expression can override the influence of FAAH (Thors *et al.*, *PLoS ONE* 5 [2010] e12275).

Interpretation of these findings in mechanistic terms has greatly been aided by a recent study investigating signalling pathways in astrocytoma cells with different expression levels of CB<sub>1</sub> receptors: at moderate expression levels, activation of the receptors leads to apoptosis, whereas at high levels, the receptors couple additionally to the pro-survival factor Akt and apoptosis is not found unless this additional pathway is inhibited (Cudaback *et al.*, *PLoS ONE* 5 [2010] e8702). Our data are consistent with the hypothesis that a signalling switch like this

also occurs in prostate cancer. Thus, at moderate CB<sub>1</sub> expression levels, a high FAAH expression would reduce local anandamide levels and hence its potential protective effect. At high CB<sub>1</sub> expression levels, the pro-survival pathway would render the cells resistant to the beneficial effects of the local endocannabinoid environment, and thereby result in a poorer prognosis for the patient. Further studies are currently in progress to determine how the CB<sub>1</sub> receptor expression level interacts with other disease biomarkers.

**NOTAS:**

## Premio a la Mejor Publicación 2010

### MICROGLIAL CB<sub>2</sub> CANNABINOID RECEPTORS ARE NEUROPROTECTIVE IN HUNTINGTON'S DISEASE EXCITOTOXICITY

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Cannabinoid-derived drugs are promising agents for the development of novel neuroprotective strategies. Activation of neuronal CB<sub>1</sub> cannabinoid receptors attenuates excitotoxic glutamatergic neurotransmission, triggers prosurvival signalling pathways and palliates motor symptoms in animal models of neurodegenerative disorders. However, in Huntington's disease there is a very early downregulation of CB<sub>1</sub> receptors in striatal neurons that, together with the undesirable psychoactive effects triggered by CB<sub>1</sub> receptor activation, foster the search for alternative pharmacological treatments. Here, we show that CB<sub>2</sub> cannabinoid receptor expression increases in striatal microglia of Huntington's disease transgenic mouse models and patients. Genetic ablation of CB<sub>2</sub> receptors in R6/2 mice, that express human mutant huntingtin exon 1, enhanced microglial activation, aggravated disease symptomatology and reduced mice lifespan. Likewise, induction of striatal excitotoxicity in CB<sub>2</sub> receptor-deficient mice by quinolinic acid administration exacerbated brain oedema, microglial activation, proinflammatory-mediator state and medium-sized spiny neuron degeneration. Moreover, administration of CB<sub>2</sub> receptor-selective agonists to wild-type mice subjected to excitotoxicity reduced neuroinflammation, brain oedema, striatal neuronal loss and motor symptoms. Studies on ganciclovir-induced depletion of astroglial proliferation in transgenic mice expressing thymidine kinase under the control of the glial fibrillary acidic protein promoter excluded the participation of proliferating astroglia in CB<sub>2</sub> receptor-mediated actions.

**NOTAS:**

**CB<sub>1</sub> CANNABINOID RECEPTOR DISTRIBUTION IN RAT CEREBELLUM DURING EARLY POSTNATAL DEVELOPMENT**

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The cerebellar cortex is subjected to morphological changes during postnatal development. The cannabinoid type 1 (CB1) receptor is expressed in parallel and climbing fibres, the principal excitatory inputs of Purkinje cells, as well as in inhibitory synaptic terminals. Those synapses are established at different postnatal times during the first three weeks. Although it is known that CB1 is present from P14 to adult in the molecular layer of the mouse cerebellum (Kawamura et al., 2006) there are not so far anatomical studies carried out at earlier postnatal stages. The goal of this investigation was to determine the CB1R distribution pattern in the cerebellar cortex in parallel fibres at postnatal day 5 (P5) to compare it with the CB1R pattern at P12. We have performed a double labelling immunocytochemical approach for confocal microscopy localizing CB1R in compartments identified as parallel fibres by the vesicular glutamate transporter type 1 (VGluT1). At both studied ages the molecular layer showed a profuse colocalization of CB1R with VGluT1 suggesting the presence of CB1R in parallel fibres. Taking into account the presence of CB1R in axons of granule cells at P5 and P12 we performed high resolution preembedding immunoelectron microscopy experiments to determine the precise localization of CB1R in these profiles at these early stages of rat postnatal development. The expression pattern of CB1R observed at P5 and P12 was mainly restricted to the parallel fibres in the molecular layer. Therefore, immunopositive profiles for CB1R increased during development in synaptic terminals and preterminals, while it was decreased in axons. These results show a differential localization of the CB1R at early stages of postnatal development suggesting the involvement of this receptor in cerebellar maturation and function.

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**NOTAS:**

## **INTRINSIC MECHANISMS OF THE PRODUCTION OF 2-AG AND AEA MEDIATING DIFFERENT FORMS OF SYNAPTIC PLASTICITY IN A SINGLE NEURON**

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We have previously shown that the production of the endocannabinoids (eCB) 2-arachidonoylglycerol (2-AG) and anandamide (AEA) in a single neuron is controlled by distinct stimuli and that they mediate different forms of synaptic plasticity in the bed nucleus of the stria terminalis (BNST).

We have seen that a postsynaptic depolarization of a BNST neuron triggers short-term depression (STD) through L-Type Ca<sup>2+</sup> channels and 2-AG, whereas synaptic stimulation triggers long-term depression (LTD) through the metabotropic glutamate receptor mGluR5 and AEA. Here we report the intrinsic mechanisms of the production of each endocannabinoid.

First, we have tested how 2-AG is synthesized after a brief depolarization of a postsynaptic BNST neuron to induce STD. For that purpose, bath application of U73122, a specific phospholipase C (PLC) inhibitor, abolishes BNST STD. Taken together, a postsynaptic depolarization elicits STD through Ca<sup>2+</sup> influx via L-type Ca<sup>2+</sup> channels, PLC activation and 2-AG generation, which acts on presynaptic CB1R.

Second, bath application of U73122 does not affect LTD, indicating that PLC activation is not required for LTD induction in BNST. Then, we have evaluated the Ca<sup>2+</sup> requirements for LTD. The Ca<sup>2+</sup>-ATPase inhibitor thapsigargin, that depletes the intracellular Ca<sup>2+</sup> stores, blocks eCB-LTD. Thus, induction of BNST LTD requires postsynaptic activation of mGluR5 and a Ca<sup>2+</sup> release from intracellular stores that promotes AEA synthesis that diffuses to activate CB1R.

In conclusion, these results clarify why neurons synthesize more than one type of endocannabinoid. Furthermore, the involvement of AEA and 2-AG in distinct physiological processes will pave the way for selective enzyme inhibitors to dissect the beneficial and harmful effects of cannabinoid receptor activation.

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**NOTAS:**

**ENDOCANNABINOIDS POTENTIATE SYNAPTIC TRANSMISSION THROUGH ASTROCYTE STIMULATION**

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We have investigated the effects of the ECB-mediated neuron-astrocyte signalling on synaptic transmission.

Using electrophysiological and  $\text{Ca}^{2+}$  imaging techniques in mouse hippocampal slices, we performed paired recordings from CA1 pyramidal neurons and simultaneously monitored astrocyte  $\text{Ca}^{2+}$  levels. We stimulated Schaffer collateral single synapses using the minimal stimulation method, and we quantified the synaptic transmission properties. ECBs were released by depolarizing one pyramidal neuron (ND) while EPSCs were monitored in the adjacent neuron. We found that endocannabinoids released by hippocampal pyramidal neurons increase the probability of transmitter release at single CA3-CA1 synapses. This synaptic potentiation is due to CB1R-induced  $\text{Ca}^{2+}$  elevations in astrocytes, which stimulate the release of glutamate that activates presynaptic group I metabotropic glutamate receptors. While endocannabinoids induce  $G_{i/o}$  protein-mediated homosynaptic depression by activation of presynaptic CB1Rs, they lead to heterosynaptic potentiation by activation of CB1Rs in astrocytes.

We conclude that endocannabinoids potentiate excitatory synaptic transmission through stimulation of  $\text{Ca}^{2+}$ -dependent release of glutamate from astrocytes.

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**NOTAS:**

**CB<sub>1</sub> CANNABINOID RECEPTORS REGULATE PYRAMIDAL NEURON SPECIFICATION**

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CB<sub>1</sub> receptors, the main molecular targets of psychoactive compounds of *Cannabis Sativa* and the endogenous ligands 2-AG and anandamide, exert a crucial neuromodulatory role in the central nervous system. Besides, due to their ability to activate pro-survival signaling cascades, CB<sub>1</sub> receptors are protective against neuronal degeneration and contribute to the control of the generation and survival of neural cells. Recent studies have unravelled a previously unknown role for CB<sub>1</sub> receptors during mammalian brain development. Here we investigated whether CB<sub>1</sub> cannabinoid receptors regulate pyramidal neurogenesis and cortical layer specification. We found that CB<sub>1</sub> receptor deletion reduced Pax6- and Tbr2-positive progenitor cell number in the ventricular and subventricular zone, and induced an expansion of post-mitotic Tbr1- and Satb2-positive cells as a consequence of precocious cell cycle exit and commitment of progenitors. Conditional CB<sub>1</sub> receptor ablation in the glutamatergic lineage, but not in the GABAergic lineage, reduced Ctip2 projection neurons, which supports a pyramidal lineage-autonomous impact of CB<sub>1</sub> receptors in deep layer specification. Axonal fasciculation deficits in CB<sub>1</sub>-deficient mice impaired skilled-motor function, thus reflecting corticospinal connectivity deficits. These findings show that CB<sub>1</sub> cannabinoid receptors, by controlling some elements of the transcriptional regulatory network responsible for cortical development, coordinate progenitor cell cycle with laminar specification in the mouse developing cortex.

**NOTAS:**

## EFFECTS OF NEONATAL MATERNAL DEPRIVATION AND/OR A HIGH FAT DIET ON HYPOTHALAMIC AND HIPPOCAMPAL ENDOCANNABINOID LEVELS IN MALE AND FEMALE RATS

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We have previously shown that maternal deprivation (MD) in neonatal Wistar rats (24 hours at PND 9) induces short- and long-term metabolic and endocrine effects including a reduction in plasma leptin levels (Viveros et al *Horm Behav.* 2010, 57:405-14; and *Horm Behav.* 2010 Aug 11. [Epub ahead of print]), as well as sex-dependent modifications in the hippocampal endocannabinoid system (Llorente et al *Psychoneuroendocrinology.* 2007, 32:636-50; Viveros et al *Psychoneuroendocrinology.* 2009, 34 Suppl 1:S217-26; Suarez et al *Hippocampus.* 2009, 19:623-32. and *Brain Res.* 2010,1349:162-73). In view of the crucial role of the endocannabinoid system in energy balance (Viveros et al, *Endocr Metab Immune Disord Drug Targets.* 2008, 8:220-30; Bermudez-Silva et al *Pharmacol Biochem Behav.* 2010, 95:375-82), we have now evaluated the effects of MD and/or a high fat diet (HFD) [D12450B (10% fat) as control and D12451 (45% fat) as HFD (Research Diets, Brogaarden)], from weaning [postnatal day (PND) 22] until adulthood (PND 101) on the levels of anandamide (AEA), 2-arachidonoylglycerol (2-AG), palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) in the hypothalamus and hippocampus of adult male and female Wistar rats, as well as on body weight. Animals were sacrificed at PND 102, beginning at 09:00h, after a 12h fasting period (from 21:00 of PND 101). Tissue levels of endocannabinoids and *N*-acylethanolamines were measured using high performance liquid chromatography coupled with tandem mass spectrometry on a triple quadrupole instrument (Agilent 6460). Male MD rats on a normal diet showed significantly lower levels of PEA in the hypothalamus compared with female MD rats on a normal diet. MD rats (male and female) on a high fat diet had higher levels of 2-AG in the hippocampus, compared with non-MD rats on the same high fat diet. MD male rats, but not MD female rats, on a normal diet, had reduced levels of PEA in the hippocampus, compared with non-MD controls. MD, “per se” induced a decrease in body weights throughout the experimental period, whereas the special high fat diet counteracted this effect. Moreover, at adulthood, MD males on the high fat diet weighed more than control non deprived males receiving the same special diet. Our results support the idea that MD and a diet-induced metabolic challenge may affect endocannabinoids and related lipids not only in brain regions directly involved in metabolic regulation but also in other brain regions mediating motivational and cognitive processes (Massa et al *J Neurosci.* 2010, 30:6273-81). Moreover, the data represent further validation of MD as a useful animal model to investigate metabolic disorders with a neurodevelopmental origin.

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**NOTAS:**

## STUDY OF THE POLYGENIC INFLUENCE ON THE CANNABINOID TETRAD TESTS USING CONSONOMIC MICE STRAINS

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Consonomic strains, also known as chromosome substitution strains, are powerful tools for assigning polygenes that control quantitative complex traits to specific chromosomes. Cannabinoids produce a characteristic profile of *in vivo* effects in mice: suppression of spontaneous activity, antinociception, hypothermia, and catalepsy; as a result of the cannabinoid tetrad tests. Despite that these behaviours are modulated by the activation of CB1 receptors, codified by the *Cnr1* gene located in chromosome 4 in mice, we have decided to explore other chromosomes that could be implied in such *in vivo* effects in mice. For this purpose we have used mouse consonomic strains and the cannabinoid receptor agonist WIN55,212-2. The inbred mice strain C57BL/6J was used as the host background and the inbred mice strain MSM/Ms (which is derived from the Japanese wild mouse *Mus musculus molossinus*), as the donor strain. Every chromosome from C57BL/6J mice was replaced by its counterpart from MSM/Ms mice.

As a first step of the study, the cannabinoid tetrad tests were used in CB1 knockout (KO) mice and their respective Wild type (WT) mice controls. We found that WIN55,212-2 – induced catalepsy, -nociception, -decrease in body temperature and -immobility were reduced or virtually blocked in *Cnr1* KO mice when compared with WT mice. In the second part, in both parental strains (C57BL/6J and MSM/Ms mice) the cannabinoid tetrad tests were used again. We found that WIN55,212-2 caused significant differences between both mice strains in body temperature and immobility time, but not in catalepsy and nociception. Finally in the third part, we repeated all the tests but using consonomic animals. Here, we provide evidences that chromosomes 13C, 16, 19 are also responsible for the decrease in body temperature and motor activity after cannabinoid receptor agonist administration.

The present results suggest that the characteristic *in vivo* profile in mice after cannabinoid CB1 receptor activation is regulated also by other genes located on other chromosomes. As well, these results could be pointing some clues to reveal new genetic associations with the behaviours observed after cannabinoid receptor activation.

**NOTAS:**

**DIFFERENTIAL ROLE OF ENDOCANNABINOIDS IN MEMORY, NOCICEPTION AND ANXIETY-LIKE RESPONSES**

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Cannabinoid agonists are potential therapeutic agents due to their antinociceptive and anxiolytic-like effects, although an important caveat to their use is the possible adverse effects related to memory impairment. An alternative approach to avoid this limitation consists in enhancing the concentration of the endocannabinoids anandamide and 2-arachidonoylglycerol. Using the specific inhibitors of the endocannabinoid metabolizing enzymes fatty acid amide hydrolase, URB597, and monoacylglycerol lipase, JZL184, we show that anandamide modulation promotes deficits in memory consolidation, whereas 2-arachidonoylglycerol modulation has not such effects. This dichotomy on endocannabinoid effects is also observed in the modulation of mTOR activity in the hippocampus by URB597, resembling those of delta9-tetrahydrocannabinol, which is not observed after JZL184 administration. In contrast, both URB597 and JZL184 induced similar acute antinociceptive effects in thermal and visceral nociceptive paradigms and anxiolytic-like effects in the plus-maze and zero-maze paradigms. Interestingly, the magnitude of the effects observed after a single administration of the URB597 and JZL184 in the different behavioral paradigms were similar to those observed after a repeated treatment with these drugs demonstrating the lack of tolerance, paralleled by the lack of change in the expression of CB1 receptor in the hippocampus. These results dissociate the role of anandamide and 2-arachidonoylglycerol in memory consolidation after their acute and chronic modulation, and support the use of 2-AG degradation inhibitors as possible therapeutic tools for the management of anxiety and pain.

**NOTAS:**

**SPATIAL MEMORY IMPAIRMENT IN DEVELOPMENTAL HYPOTHYROID RATS: IMPLICATION OF THE ENDOCANNABINOID SYSTEM**

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Thyroid hormones, T<sub>3</sub> and T<sub>4</sub>, are essentials for proper brain development and function, so alterations in cognitive functions are well-characterized features of the hypothyroid phenotype, both, in humans and experimental animals. Moreover, biochemical, morphological and electrophysiological data showing that the hippocampus is a target of thyroid hormones. In the present study, we have analyzed the effect of congenital hypothyroidism on spatial memory and hippocampal sensitivity to cannabinoid agonists. Here we show that adult hypothyroid rats display a lowest performance than control rats in the Morris water maze, and that their performance is worsened by the cannabinoid agonist WIN 55212-2 at a dose of 0.2 mg/kg, which had no effect on the performance of control rats. On the other hand, an increased biochemical response to cannabinoids was observed in the hippocampus of hypothyroid rats, as determined by ERK1/2 phosphorylation and c-fos and egr-1 genes expression. Furthermore, no changes were observed in the levels of CB1 receptor protein in the hippocampus of hypothyroid rats. These effects of developmental hypothyroidism are essentially irreversible, since the treatment of adult hypothyroid animals with thyroid hormones did not improved Morris water maze performance.

**NOTAS:**

**CHRONIC DELTA9-TETRAHYDROCANNABINOL AFFECTS CEREBELLAR FUNCTION THROUGH THE ACTIVATION OF CEREBELLAR MICROGLIA**

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Heavy cannabinoid exposure has been associated to cerebellar dysfunction in humans. On the other hand, it has been previously demonstrated a relation between proinflammatory cytokines and the induction of the cerebellar ataxia. We observe using immunofluorescence analysis that chronic administration of delta9-tetrahydrocannabinol (THC) in mice modifies both the expression of the microglial activation marker CD11b and the microglial cell morphology in the cerebellar cortex. In these conditions cerebellar CB1 receptors were clearly down-regulated while CB2 receptor expression did not change. Five days after the spontaneous withdrawal of THC, cerebellar microglial population remained in the activated state. Furthermore, cerebellar CB1 receptor expression was still down-regulated while CB2 receptor expression was up-regulated. Subtle motor coordination deficits were observed by the coat-hanger test five days after the end of the THC-chronic treatment. To further relate these subtle deficits to a possible change in cerebellar function we studied the delayed eye-blink conditioning as a direct measurement of cerebellar function. Mice chronically treated with THC showed a clear deficit in this test. Interestingly, inhibition of microglial activation by minocycline during the THC withdrawal period reduced the characteristic markers of microglial activation and reversed the locomotor coordination deficits associated to the cannabinoid withdrawal. Moreover, performance in the delayed eye-blink conditioning test was significantly improved in THC-withdrawn mice after minocycline chronic treatment, revealing the activation state-dependent modulatory role of microglial cells in cerebellar function. Finally, we observe that chronic THC administration altered the expression of cerebellar glial glutamate transporter EAAT1/GLAST. We therefore hypothesize that the change in glutamate function in the cerebellum resulting of chronic THC exposure would produce the activation of microglial cells in the cerebellar cortex, underlying a subtle long term deficit in cerebellar dependent functions.

**NOTAS:**

**CANNABINOID RECEPTOR I (CB<sub>1</sub>) RECEPTORS ARE PRESENT IN PANCREATIC POLYPEPTIDE AND DELTA CELL SUBPOPULATIONS WITHIN MOUSE PANCREATIC ISLETS**

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The appetite-stimulating effects of marijuana (*Cannabis sativa*) have been known since ancient times. Delta(9)-tetrahydrocannabinol (THC), the main psychoactive component of marijuana, increases food intake through activation of CB1 cannabinoid receptors and this effect makes the CB1 receptor an interesting therapeutic candidate in cancer- and HIV-related cachexia. However, the exact mechanism by which CB1 promotes food intake up to date is not fully understood. We hypothesize that peripheral CB1 receptors may participate in the appetite-increasing properties of THC. The endocrine pancreas is in charge of metabolic homeostasis and it is formed of pancreatic islets, mainly composed of four cell types, alpha-, beta-, delta-, and pancreatic polypeptide cells (PP). Here we evaluated the presence of CB1 receptors in the mouse pancreas by immunofluorescence. Our results revealed that a subpopulation of mouse pancreatic PP cells and delta cells express CB1 receptors. In a much lesser extent, beta cells also showed some CB1 receptor signal. As expected, pancreatic islets from CB1 knockout mice lacked the CB1 expression present in wild-type animals. Pancreatic polypeptide, secreted by pancreatic islet PP cells is known to suppress food intake by acting on central receptors and by inhibition of gastric emptying. This polypeptide is currently in the spotlight, and it is being evaluated clinically, as a putative target for the treatment of obesity and other food-intake related disorders. This is the first study showing presence of CB1 receptors in pancreatic polypeptide cells. We hypothesize that inhibition of pancreatic polypeptide release by CB1 receptors may plausibly contribute to THC-enhanced appetite and the appetite-suppressing actions of CB1 receptor antagonists.

**NOTAS:**

**ROLE OF THE CB<sub>2</sub> CANNABINOID RECEPTOR IN ERBB2-DRIVEN BREAST CANCER PROGRESSION**

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It is well known that cannabinoids exert antitumoral effects in different models of cancer. They inhibit cancer cell proliferation, adhesion, migration and induce cell death by apoptosis. However, little is known about the role of the endocannabinoid system in tumor physiology. In particular, although strong evidence point to the CB<sub>2</sub> cannabinoid receptor as target for anti-cancer therapy, there is no information about its role in tumor generation and progression.

To shed light on this issue, we have generated animals with two genetic modifications, specifically, Her2 overexpression directed to the mammary epithelium, which triggers the spontaneous generation of breast tumors, and genetic ablation of the CB<sub>2</sub> cannabinoid receptor. These double transgenic animals were analyzed in terms of tumor latency, tumor growth, number of tumors generated per animal and development of lung metastases.

Our results show that the lack of CB<sub>2</sub> receptors has a protective effect on tumor generation and progression. We are currently analyzing the molecular mechanisms involved in this tumor phenotype.

**NOTAS:**

## **CANNABINOID-BASED COMBINED THERAPIES AS A NOVEL STRATEGY TO FIGHT GLIOMAS?**

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$\Delta^9$ -Tetrahydrocannabinol (THC), the major active ingredient of marijuana and other cannabinoid receptor agonists inhibit tumor growth in animal models of cancer, including glioma, an effect that relies, at least in part, on the stimulation of autophagy-mediated apoptosis in tumor cells. Glioblastoma multiforme (GBM) is highly resistant to current anticancer treatments which makes crucial to find new therapeutic strategies aimed at improving the poor prognosis of patients suffering this disease. The identification of the molecular mechanisms responsible for the resistance of gliomas to anti-cancer treatments is an issue of great therapeutic interest. In this work we identified a subset of genes specifically associated to THC-resistance by analyzing the gene expression profile of human glioma cells with different sensitivity to cannabinoid-induced cell death. We found that one of these genes, the secreted growth factor midkine (Mdk) is directly involved in the resistance of glioma cells to cannabinoid treatment. We also show that Mdk mediates its protective effect via the anaplastic lymphoma kinase receptor (ALK) and that Mdk signaling through ALK interferes with cannabinoid-induced autophagic cell death. Furthermore, in vivo Mdk silencing or pharmacological inhibition of ALK sensitizes cannabinoid-resistant tumors to THC anti-tumoral action.

In addition, we investigated whether the combined administration of cannabinoids and other antitumoral agents could be beneficial in glioma therapies. To this aim, we selected THC, Cannabidiol and Temozolomide (an alkylating agent that is currently used for the treatment of malignant gliomas). Results show that the combined administration of THC, CBD and TMZ exerts a strong anti-tumoral action in glioma xenografts, an effect that is also observed in tumors that are resistant to TMZ treatment.

Altogether, our findings support that the combined administration of cannabinoids and TMZ and/or selective inhibitors of the ALK tyrosine kinase receptor could be therapeutically exploited for the management of GBM.

**NOTAS:**

## **CANNABIS-OIL EXTRACTION: RELATED CASE**

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THC has demonstrated to induce apoptose and inhibition of neoangiogenese in some tumoral cellular lines.

In this apresentation the results obtained in a 92 years old male patient treated for a face basalioma with a cannabis-oil extract are showed.

The treatement was made with 6 intratumoral inyections of a based-cannabis extract (absolute alcool pre-dilution)

The pre-treatement tumoral pictures compared with post-treatement tumoral pictures encourage us make more clinical experiences in the future.

**NOTAS:**

## GABA/GLUTAMATE IMBALANCE IN THE HIPPOCAMPUS OF ADULT FEMALE RATS EXPOSED TO CANNABINOIDS DURING ADOLESCENCE

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Adolescence is a period of active synaptic remodelling and plasticity and therefore of special vulnerability to the effects of drugs of abuse. Indeed, cannabinoid exposure during periadolescence and adolescence has been linked to long lasting alterations in learning and memory processes as well as alterations in hippocampal neuronal morphology; however, the neural changes underlying such effects remain poorly understood. Here we show that adult Wistar rats of both sexes chronically treated with the cannabinoid agonist CP 55,940 (CP) during adolescence exhibit increased K<sup>+</sup>-induced GABA release in the CA1 hippocampal field as well as decreased GABA transporter gene expression. Together with this effect, CP-females also showed increased GABA<sub>A</sub> receptor expression, decreased K<sup>+</sup>-induced glutamate release and decreased NMDA receptor expression. No differences in glutamate transporters (glial -EAAT2-, or neuronal -EAAT3) were evident between CP- and VH-treated animals or between males and females. No changes in the levels of mGluR5 receptors were either observed. In order to examine the intracellular signalling downstream of mGluR5 activation we also examined the levels of ERK and its phosphorylated form in nuclear or cytoplasmic hippocampal extracts. Decreased nuclear phosphorylation of the 42kDa isoform of ERK was observed in CP-males. Taken together, these results suggest that early cannabinoid exposure is associated with strong inhibition in the hippocampus in the females and decreased nuclear ERK signalling in the males. These changes may provide a neuronal basis of the behavioral, neurophysiological and morphological alterations found after early cannabinoid exposure.

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**NOTAS:**

## A STUDY ON WIN 55,512-2 SELF-ADMINISTRATION BEHAVIOUR IN LEWIS AND FISCHER 344 RATS

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There is controversy in the literature as to what extent cannabinoids may have addictive potential. While non-human primates self-administer of  $\Delta$ -9 Tetrahydrocannabinol and anandamide, the results are less robust in the rat. Indeed, only the synthetic cannabinoids WIN 55,512-2 (WIN) and CP 55,940 seem to be self-administered by rats. The Fischer 344 (F344) and Lewis inbred rat strains have been used as a model to study the role of genetic factors in drug reinforcement. Indeed, in spite of being histocompatible and sharing most of their genetic makeup, LEW rats show greater self-administration of most drugs of abuse than F344 rats. The present experiments were devised with the purpose of studying self-administration behaviour of the cannabinoid agonist WIN in LEW and F344 rats. Animals were kept at 95%-90% of their free feeding weight and WIN was self-administered at a dose of 12.5  $\mu$ g/kg in a fixed ratio 1 schedule of reinforcement. Sessions lasted 1 hour during the first week and two hours for the remaining of the experiments. On the twentieth session, the effects of the CB1 antagonist/inverse agonist AM251 (4 mg/kg i.p.) on WIN self-administration were studied. After two additional sessions, WIN was substituted by vehicle and rats were given 5 extinction sessions. No robust self-administration behaviour was evident in any of the strains, although a clear preference for the active lever was observed both in LEW and F344 rats. Interestingly, AM251 challenge reduced active lever pressing only in F344 rats. Rats of both strains also failed to extinguish their lever-pressing behaviour. In order to study the endocannabinoid system in these two rat strains, cannabinoid receptor distribution and levels were studied by quantitative autoradiography of [<sup>3</sup>H]-CP 55,940 binding in brain slices that included the basal ganglia, limbic system, ventral tegmental area, substantia nigra and cerebellum. Strain differences were detected only in the lateral globus pallidus where F344 showed higher binding levels than LEW rats. In conclusion, although no clear WIN self-administration behaviour was observed, a preference for the active lever was evident, pointing to a certain degree of reinforcement elicited by the cannabinoid which would, in the case of F344 rats, depend on CB1 receptors, perhaps at the level of the lateral globus pallidus. These results corroborate those previously reported in the literature that suggested that in rats, cannabinoids might be devoid of addictive properties.

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**NOTAS:**

**COCAINE SELF-ADMINISTRATION DIFFERENTIALLY MODULATES ENDOGENOUS CANNABINOID SYSTEM PROTEINS IN THE RAT HIPPOCAMPUS OF FISHER AND LEWIS RATS**

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Glutamate and GABA are two transmitters involved on the acquisition and relapse to cocaine self-administration. The endocannabinoids anandamide and 2-AG are modulators of glutamate and GABA synapses in the brain but little is known on the effects of this psychostimulant on the enzymes that produce and degrade endocannabinoids. The present work addresses the effects of cocaine self-administration on the expression of endocannabinoid signalling proteins in the hippocampus of two different strains of rats, Lewis (LEW) and Fischer 344 (F344), which differ in their response to drugs of abuse and they have frequently been used as an experimental model to study the vulnerability to drug addiction. There were only subtle strain differences in the expression of cannabinoid CB1 receptors in the hippocampus of LEW and F344 rats. After cocaine self-administration training, both strains showed clear differences on the expression of endocannabinoid signalling proteins. Whereas Lewis rats showed a decrease in the NAPE-PLD/FAAH ratio, suggesting decreased production of the endocannabinoid anandamide, F344 rats showed higher DAGLalpha/MAGL ratios, indicating higher 2-AG generation. These findings indicate that cocaine may modulate hippocampal GABA/Glutamate synapses by directly modulating endocannabinoid production enzymes, and that these actions are strain-dependent. Thus, the endogenous cannabinoid system contributes to individual/strain differences on the vulnerability to drug abuse.

**NOTAS:**

## MATERNAL DEPRIVATION EFFECTS ON MDMA INDUCED CONDITIONED PLACE PREFERENCE IN ADOLESCENT RATS AND SEX-DEPENDENT CHANGES IN HIPPOCAMPAL CB<sub>1</sub> RECEPTOR

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We have previously shown that maternal deprivation (MD) in neonatal Wistar rats (24 hours at PND 9) induces sex-dependent modifications in the hippocampal endocannabinoid system (decreased CB<sub>1</sub> receptor expression and 2-AG levels) (Llorente R et al., *Psychoneuroendocrinology*. 2007, 32:636-50; Viveros et al., *Psychoneuroendocrinology*. 2009, 34 Suppl 1:S217-26; Suarez et al., *Hippocampus*. 2009, 19:623-32. and *Brain Res*. 2010,1349:162-73) as well as marked modifications in the serotonergic system of diverse brain regions in adolescent MD rats (Llorente et al *Neurosci Lett*. 2010, 479:112-7 ). In view of the potential role of the cannabinoid system in the rewarding effects of 3,4-methylenedioxymetamphetamine (MDMA) (Manzanedo et al., *Behav Brain Funct*. 2010, 22:6:19) and the reported effects of MDMA on the serotonergic system (Wallinga et al *Pharmacol Biochem Behav*. 2009, 94:227-33), we hypothesized that MD would alter the neurobiological consequences of MDMA. In this study we analyzed the effects of MD on MDMA induced conditioned place preference (CPP) during the adolescent period. The CPP consisted of three phases that were carried out at the following ages: PND34, acquisition; PND 35-41, pre-conditioning and PND 42, post-conditioning. Rats received an injection of 2.5 mg/kg MDMA (3,4-methylenedioxymetamphetamine hydrochloride, Sigma-Aldrich, Spain) immediately before confinement in the drug-paired compartment for 30 min on days 2, 4, 6, and 8 (PN D 35, 37, 39, and 41) and physiological saline before being confined to the vehicle-paired compartment for 30 min on days 3, 5, 7 and 9. Animals were sacrificed at PND 68-75 and the expression of hippocampal CB<sub>1</sub> receptors (Western Blot) as well as the plasma levels of corticosterone (radioimmunoassay) and leptin (ELISA) were determined. The results indicated that control non MD males spent significantly more time in the MDMA associated compartment during the postconditioning phase than in the preconditioning phase, whereas this was not the case in the MD males. Accordingly, the time spent during the postconditioning phase in the MDMA associated compartment was significantly longer in control males than in MD males. The dose of MDMA used did not induce CPP in females. MDMA treated MD males showed an increased expression of hippocampal CB<sub>1</sub> cannabinoid receptor and a strong tendency to elevated circulating leptin levels when compared to control non deprived males, whereas these MDMA effects were not observed in MD females. In addition to these sexual dimorphisms, additional sex differences were found in corticosterone and leptin levels with males showing significantly more leptin and less corticosterone than females.

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**NOTAS:**

## EFFECTS OF CANNABIS CONSUMPTION IN SUBCLINICAL NEGATIVE PSYCHOTIC EXPERIENCES

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*Research aim:* Our purpose was to establish if cannabis could increase the probability to suffer the negative psychotic symptoms in healthy people which never received psychiatric assistance. We take account the frequency and the initial age of drug-taking

*Methodology:* The participants were recruited by means of “snow ball” method. We divided the participants in two groups; cannabis daily consumers (148 participants) or not daily consumer (110 participants; the use frequency was lower or nonexistent). We distributed this sample in five levels to analysis the data; (i) cannabis monthly consumers [38 participants, once or twice per month]; (ii) cannabis weekly consumers [44 participants, more that twice per months and less than daily consumer]; (iii) cannabis daily consumers [84 participants, between once and four times per day]; (iv) five or more times per day [64 participants]; (v) No cannabis consumers [28 participants, less than five times along their life]. The expression of positive psychotic symptoms was assessment with Community Assessment of Psychic Experiences (CAPE). We measured the probability to show one, two or three positive psychotic symptoms “often” or “nearly always”.

*Results:* General results showed the daily consumers had a higher probability to show negative psychotic symptoms compared with less daily consumers (logistic regression). Nevertheless, when we took account the sex, age, alcohol consume and another psychoactive substance consume we did not obtain results for two symptoms. Only obtain significative interaction for three symptoms. The logistic regression between the five use frequency groups showed higher incidence for three symptom in five or more times per day consumers. Beginning consumers (They taste cannabis younger than fourteen) don't showed higher probability to express negative psychotic symptoms

*Conclusions:* Daily use of five or more cannabis cigarettes can increase five times the expression of negative psychotic symthoms after to took account the sex, age, alcohol consume and another psychoactive substance consume.

**NOTAS:**

**HUMAN SINGLE NUCLEOTIDE POLYMORPHISMS (SNP'S) FROM THE CANNABINOID AND CATECHOLAMINERGIC SYSTEM ARE ASSOCIATED TO DRUG CONSUMPTION AND BASIC PSYCHOLOGICAL PROCESSES**

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Vulnerability to addictive behaviors is influenced by genetic factors, such as single nucleotide polymorphisms (SNP's). In this way, some SNP's of the cannabinoid receptor 1 gene (CNR1) have been associated with marijuana abuse, whereas other SNP's of the dopaminergic receptor D2 (DRD2) have been related to cocaine addiction.

In our study we have tried to explore the association between several SNP's, which have been previously demonstrated to induce alterations in the cannabinoid and catecholaminergic system, with drug consumption and basic psychological processes. For this purpose, we sequenced the DNA from 82 samples taken from voluntary students of the Universidad Complutense de Madrid (Madrid, Spain), and assessed their performance on different behavior and cognitive tasks. Together, the heart rate response to a stress stimulus and their drug consumption habits were assessed. The results showed a significant association between some analyzed SNP's, drug consumption and basic psychological processes like memory, executive functions and motor learning. These results are in line with the presence of CB1 receptors in hippocampus, basal ganglia and cerebellum (thereby the implication of the cannabinoid system in memory, motor learning and executive functions). Furthermore, heart rate measures in baseline and in response to a stress stimulus were also related with the analyzed SNP's of the catecholaminergic system.

**NOTAS:**

## WIN 55,212-2 ATENUATES BRAIN DAMAGE AFTER PERINATAL HYPOXIC-ISCHEMIC INJURY

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**Introduction:** Cannabinoids have shown therapeutic potential in cerebral ischemic disorders and have been proposed as neuroprotective agents against the brain damage produced during perinatal asphyxia, which remains as a major cause of neonatal morbidity and mortality. The aim was to evaluate the effect of the cannabinoid agonist WIN 55,212-2 in the brain of premature fetal lambs analyzing necrosis, apoptosis and some intracellular fate parameters after hypoxic-ischemic (HI) injury induced by partial occlusion of the umbilical cord.

**Methods:** 18 fetal lambs were randomly assigned to: one control group and two HI groups, one receiving i.p. a single dose of 0.01 µg/kg of WIN just after injury (induced by partial occlusion of the umbilical cord during 60 minutes). All lambs were maintained with mechanical ventilation for 3 hours and then sacrificed. Non-fixed brains were divided into different brain regions, disaggregated and cell suspensions analyzed by flow cytometry for cell viability and intracellular fate studies. One-factor ANOVA was performed (p<0.05).

**Results:** The number of necrotic cells was similar in both HI groups. The apoptosis index was significantly increased in the HI group in comparison with the control, but the administration of the cannabinoid agonist prevented that increase. WIN treated animals showed similar values of mitochondrial integrity and membrane potential in comparison with those observed in control group, whereas there was a loss of mitochondrial functions in the HI group. This group also showed a significantly higher concentration of oxygen reactive species and intracellular calcium in comparison to control, but in this case, WIN only could revert these adverse effects in some brain regions.

**Conclusion:** Our results suggest that the administration of the cannabinoid agonist WIN 55,212-2 after hypoxic-ischemic brain injury in preterm lambs does not discard death by necrosis, but maintains apoptotic levels and some intracellular parameters similar to control, suggesting that WIN treatment may have potential therapeutic benefits after the HI event.

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**NOTAS:**

## STUDY OF THE ADMINISTRATION OF 2-ARACHIDONYLGLYCEROL AND ANANDAMIDE ON MITOCHONDRIAL STATE, GLIAL RESPONSE AND CELL DEATH IN A HYPOXIC-ISCHEMIC RAT MODEL

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When a hypoxic-ischemic [HI] event occurs, the neurons of the central nervous system are the most vulnerable elements; however, other cellular systems, such as glial cells, are affected too. As a result of this insult, different molecular mechanisms can be enhanced whose final result is promoting cellular death. Nowadays, several research lines are focused on the study of endocannabinoid substances as a neuroprotector therapy to prevent the damage.

The aim of the present work is to study the effect of the administration of AEA and 2-AG cannabinoid substances on brain damage after a HI injury.

We have used the Rice–Vannucci experimental procedure to cause HI brain injury in 7-day-old Wistar rats. Immediately after the HI event an intraperitoneal injection of endocannabinoids, AEA [5mg/Kg] and 2-AG [1mg/Kg], were administrated. The brains were analysed 24 h, 72 h and 7 days after the HI injury. For the study of ipsilateral brain region with flow cytometry we used: non-fixed tissue samples for mitochondrial transmembrane potential and apoptotic cells, and fixed tissue samples for the mitochondrial integrity and GFAP expression.

Mitochondrial transmembrane potential, tested with Rhodamine 123, exhibited a high decrease of positive cells at 24 h in all injured groups; and endocannabinoid treated groups failed to recover this situation. The mitochondrial integrity study, testing the cardiolipin, with Nonyl Acridine Orange, showed a significative and important decrease 24h after the damage in all injured groups. However, at 72h 2-AG and AEA groups had a considerable amelioration regarding to the mitochondrial integrity. When we analysed the asymmetry of the cell membranes [Annexin V/ Propidium Iodide] an increase in the number of apoptotic cells in the HI group was observed in all times studies. In the treated groups, at 72h the percentage of apoptotic cells begins a slight decline in the treated groups, and after 7 days had a recovery as control group. On the other hand, the glial protein expression showed an increased level as soon as 24h in all injured groups, and this increase was maintained in treated groups after 72 h and 7 days. Despite the remarkable increase on the GFAP expression after the HI, after 7 days the treated animals showed a percentage of GFAP-positive cells similar to the control group.

In conclusion our results show that the HI event induces in the first 24 h, a mitochondrial failure, an increase in apoptotic cells and in GFAP expression. Administration of 2-AG and AEA, at 7 days, promote a remarkable amelioration of this cell death and a decrease of the

percentage of GFAP positive cells although with an increased expression of this protein, respect to HI group.

**NOTAS:**

## HISTOLOGICAL NEUROPROTECTION OF CANNABIDIOL AS FUNCTION OF TIME IN HIPOXIC-ISCHEMIC ENCEPHALOPATHY

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The non-psychoactive phytocannabinoid Cannabidiol (CBD) is neuroprotective for immature brain as previously described. We evaluated whether CBD neuroprotection was sustained for several days in a neonatal model of hypoxic-ischemic encephalopathy. Thus, CBD 0.1 mg/kg (HI+CBD) or vehicle (HI+VEH) was administered iv to newborn piglets 15 min after an acute hypoxic-ischemic (HI) insult (bilateral carotid artery clamping plus FiO<sub>2</sub> 10% for 20 min); non-asphyxiated piglets served as controls (SHAM). Coronal sections (4 μm) were cut and mounted on a glass slide to be stained. To quantify early neuronal necrosis, consecutive pairs of brain sections were stained by Nissl staining. To determine the presence of apoptotic cells, brain sections were stained with TUNEL. Finally, GFAP+ cells were identified in the same areas by immunohistochemistry. The concentration of neuronal specific enolase (NSE) and S100B protein were measured by ELISA in CSF samples.

At 6 h after HI, Nissl staining of brain slices revealed a decrease in the number of apparently normal neurons in cortex of HI+VEH in comparison to SHAM group (371±21 vs. 653±6 cells/mm<sup>2</sup>). In HI+CBD the loss of normal neurons was blunted and the appearance of pyknotic cells prevented; thus, normal neurons were 482±21 cells/mm<sup>2</sup>. TUNEL+ cells were not detected in any group. Also, GFAP+ cells did not show differences between groups in the number or morphology of astrocytes. Both concentrations of NSE and S100 were increased in HI+VEH in comparison to SHAM; however, the administration of CBD reduced both parameters similarly to SHAM group.

At 72 h postHI, the number of observed normal neurons in HI+VEH was lower than SHAM group (404±42 vs. 613±36 cells/mm<sup>2</sup>). However, the proportion of pyknotic cells was increased (51.8±3.5% vs. 2.3±0.4%). By contrast, in HI+CBD, the number of normal neurons in cortex (654±32 cells/mm<sup>2</sup>) and the proportion of pyknotic cells (5.4 ±1.2%) were similar to SHAM group. In cortex of HI+VEH group, the number of TUNEL+ cells was 6-fold higher of SHAM group (153.7±87.9 vs. 24.2±3.8 cells/mm<sup>2</sup>). Administration of CBD after HI reduced the number of TUNEL+ cells (46.3±10.4 cells/mm<sup>2</sup>). The number of astrocytes in HI+VEH cortex was reduced in comparison to SHAM group (92.1±3.1 vs. 110.7±7.6 cells/mm<sup>2</sup>). The surviving astrocytes appeared swollen and smaller than SHAM group (mean size 825±58 vs. 459±68 μm<sup>2</sup>). By contrast, in HI+CBD, the number of astrocytes was similar to SHAM group (116.9±5.1 cells/mm<sup>2</sup>), as well as in shape and size (mean size 703±85 μm<sup>2</sup>). In conclusion, post-HI administration of CBD led to a protective effect on neurons and astrocytes. This beneficial effect was significant sustained at least 3 days after HI.

Key Words: Cannabidiol, Hypoxic-ischemic, neuroprotection, brain.

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Institute Carlos III (grant N. RD08/0072: Maternal, Child Health and Development Network) within the framework of the VI National I+D+i Research Program (2008-2011).

**NOTAS:**

## CEREBRAL AND EXTRACEREBRAL EFFECTS OF CBD IN PIGLETS AFTER HYPOXIA-ISCHEMIA ARE MEDIATED BY CB<sub>2</sub> RECEPTORS

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*Aims and scope:* in an in vitro model of hypoxic-ischemic (HI) brain damage on newborn mice brain slices, Cannabidiol (CBD) effects were blunted by CB<sub>2</sub>R antagonists, suggesting that in immature brain and in vitro CBD effects are mediated by CB<sub>2</sub>R. The aim of the present study was to determine if CB<sub>2</sub>R are involved in CBD action in vivo and in an animal model closer to humans, as is the piglet HI model. *Methods:* HI brain damage was induced in 1-to-2 day-old piglets by occluding both carotid arteries and decreasing FiO<sub>2</sub> to 0.10 for 30 minutes. Thirty min after the end of HI, CBD 1 mg/kg (CBD, n=6) or vehicle (VHC, n=6) were administered iv. In some animals, the CB<sub>2</sub>R antagonist AM630 1 mg/kg was administered 10 min before CBD (CBD+AM, n=4). Controls were non-HI sham-operated piglets (SHM; n=4). HI effects were assessed for 6 h on brain by continuous aEEG recording; on cardiovascular system by hourly cardiac output (CO), heart rate (HR) and mean blood pressure (MBP) monitoring; and in lung by hourly dynamic compliance (C<sub>dyn</sub>) and arterio-alveolar oxygen ratio (a/AdO<sub>2</sub>) monitoring. Oxidative stress was determined in urine by measuring the concentration of isoprostanes (ELISA). *Results:* AM630 reversed all the beneficial effects of CBD on brain (aEEG at the end of the experiment -t6- 16.5±2.6, 4.4±1, 14.7±4 and 45±1 µV for SHM, VHC, CBD and CBD+AM, respct, p<0.05), cardiovascular system (changes in CO, HR and MBP: 18.9±6, -3.1±7, 4.1±7 and -7.6±5%; 25.6±10, 9.6±9, 19.8±9 and 4.9±8%; and 16.3±7, -21.1±3, -5.5±4 and -24.9±2%, for SHM, VHC, CBD and CBD+AM, respct, p<0.05) and lung (changes in C<sub>dyn</sub> and a/AdO<sub>2</sub>: 8.3±9, -31.4±9, -1.6±3 and -15.8±8%; and -6.4±0.1, -18.6±0.1, -7.6±0.1 and -19.1±0.1% for SHM, VHC, CBD and CBD+AM, respct, p<0.05). In addition, AM630 reversed the CBD inhibition of HI-induced increase of urine isoprostanes (0.3±0.1, 0.9±0.1, 0.3±0.1 and 1.3±0.6 pg/mL for SHM, VHC, CBD and CBD+AM, respct, p<0.05). *Conclusions:* in newborn animals, CB<sub>2</sub>R are involved in both cerebral and extracerebral effects of CBD.

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**NOTAS:**

## CANNABIDIOL REDUCES DISTANT LUNG INJURY SECONDARY TO HYPOXIC-ISCHEMIC BRAIN DAMAGE IN PIGLETS

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*Aims and scope:* lung damage often complicates the evolution of neonatal hypoxic-ischemic encephalopathy (HIE). The mechanisms explaining this association are not completely understood, but inflammation and oxidative stress secondary to ischemia-reperfusion injury have been implicated. No treatment but supportive care is currently available for this condition. The present study tested if Cannabidiol (CBD) reduces distant lung damage secondary to HIE and if this is due to its anti-inflammatory and antioxidant properties.

*Methods:* HI brain damage was induced in 1-to-2 day-old piglets by occluding both carotid arteries and decreasing FiO<sub>2</sub> to 0,10 for 30 minutes. Thirty min after the end of HI, CBD 1 mg/kg (CBD, n=6) or vehicle (VHC, n=6) were administered iv. Controls were non-HI sham-operated piglets (SHM; n=4). Lung function was assessed hourly during the experiment with the following parameters: arterial blood gases; dynamic compliance (C<sub>dyn</sub>; mL/cmH<sub>2</sub>O); and oxygenation index (OI=MAP x FiO<sub>2</sub> x 100/PaO<sub>2</sub>). Extravascular lung water content (EVLW), representing lung oedema, was calculated hourly using a thermodilution technique. Total protein concentration, representing lung inflammation, was determined in broncho-alveolar lavage fluid (BALF) by a BCA Protein Assay Kit at baseline and after sacrifice. Urinary levels of isoprostanes (ELISA), representing oxidative stress, were determined before sacrifice. *Results:* HI induced lung injury, as reflected by the drop of lung C<sub>dyn</sub> (-31.4±9 vs 8.3±9% in SHM), and the increase of OI (44.5±10 vs. 10.1±6%), and of EVLW (15.3±9 vs. 0.8±9%, all p<0.05). CBD administration prevented such HI-induced changes of C<sub>dyn</sub>, OI and EVLW to occur (-1.6±3%, 14±7% and -7.6±9%, respectively). Inflammation and oxidative stress were involved in HI-induced lung damage, as evidenced by the increase of BALF protein content (14.1±0.9 vs 4±0.2% in SHM) and of urine isoprostanes (0.9±0.1 vs. 0.3±0.1 pg/mL; all p<0.05). Beneficial effect of CBD was related with an antioxidant effect (urine isoprostanes 0.3±0.1 pg/mL) as well as a strong anti-inflammatory effect, as BALF protein content was even significantly lower than SHM at the end of the experiment (-0.13±0.7%). *Conclusions:* oxidative stress and inflammation are involved in HIE-induced lung injury. Post-HI CBD administration successfully prevents such lung injury by modulating both factors.

Supported by grants FIS PS09/01900 and GWCRI09119-2.

**NOTAS:**

## CANNABINOIDS AMELIORATE DISEASE PROGRESSION IN A MODEL OF MULTIPLE SCLEROSIS IN MICE, ACTING THROUGH ANTI-INFLAMMATORY AND ANTI-GLUTAMATERGIC MECHANISMS

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Multiple sclerosis (MS) is an autoimmune disease that affects the CNS and it is characterized by inflammation, demyelination, remyelination, gliosis and axonal damage, mainly occurring in the spinal cord. Cannabinoids have been proposed as promising therapeutic agents in MS given their capability to alleviate specific MS symptoms (e.g., spasticity, pain). Although MS has been considered mainly as an inflammatory disorder, recent evidence, however, revealed the importance of neurodegenerative events, opening the possibility that cannabinoid agonists, given their cytoprotective properties, may also serve to reduce oligodendrocyte death and axonal damage in MS. Thus, the treatment with WIN55,512-2, a potent CB<sub>1</sub> and CB<sub>2</sub> agonist, was reported to be effective to ameliorate tremor and spasticity in mice with chronic relapsing experimental autoimmune encephalomyelitis (CREAE), a murine model of MS, but also to delay disease progression in this and other murine models of MS. The purpose of this investigation was to further explore the mechanism(s) underlying the amelioration in disease progression caused by WIN55,212-2, particularly we have paid emphasis in anti-glutamatergic and anti-inflammatory effects of this cannabinoid agonist. In this study, we used mice treated with myelin oligodendrocyte glycoprotein that generates a progressive pattern of EAE induction and conducted the pharmacological experiments in an early stage of the disease. As expected, the administration of WIN55,512-2 (5mg/kg, i.p) had a positive effect in reducing neurological disability and improving motor coordination of EAE mice. Levels of glutamate and GABA in the spinal cord of EAE mice were similar to control animals, and, accordingly, they were not altered by the treatment with WIN55,212-2. However, EAE mice showed alterations in mRNA levels for the glutamate transporter GLT1 and, to a lesser extent, GLAST too, alterations that were reversed by the treatment with WIN55,212-2. As regards to inflammatory responses, EAE mice showed a marked up-regulation in mRNA levels for COX-2, inducible NOS and TNF- $\alpha$ , responses that were attenuated after the treatment with WIN55,212-2. We also observed the presence of cell aggregates in the spinal cord of EAE mice that were significantly attenuated by the treatment with WIN55,212-2. Immunohistochemical analysis (with Iba-1 and Cd11b) of these aggregates indicated that they corresponded to microglia (resident macrophages) and peripheral macrophages. Lastly, experiments conducted with selective antagonists for the CB<sub>1</sub> (e.g. rimonabant) or CB<sub>2</sub> (e.g. AM630) antagonists revealed that all these WIN55,212-2 effects were mediated by the activation of CB<sub>1</sub> but not CB<sub>2</sub> receptors. In summary, the treatment of EAE mice with the cannabinoid agonist WIN55,512-2 reduced their neurological disability and the progression of the disease. This effect was exerted through the activation of

CB<sub>1</sub> receptors, which would reduce excitotoxicity and also inflammation, two important events in the pathogenesis of this disease.

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**NOTAS:**

## PROTECTIVE ROLE OF CANNABINOIDS IN AN EXPERIMENTAL MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most common form of neurodegenerative disease associated with dementia in the elderly. Targeting the endocannabinoid system could offer a multi-faceted approach for the treatment of AD since several evidence indicate that cannabinoid compounds provide neuroprotection by reducing neuronal loss, neuroinflammation and oxidative stress as well as by promoting the brain's intrinsic repair mechanisms. In the present study we provide behavioural and molecular data supporting this hypothesis by using *in vivo* and *in vitro* experimental models of AD.

The chronic treatment with the CB<sub>1</sub> agonist ACEA significantly reduced the memory and learning impairment exhibited by double transgenic APP/PS1 mice at early stages of the pathology, as well as induced a slight reduction of the cortical amyloid burden and morphological changes in astroglial reactivity in these animals. In contrast, CB<sub>2</sub> agonist JWH-133 was able to improve the memory performance of transgenic mice, but not their learning capacity, and reduced the microglial reactivity associated to amyloid deposition.

*In vitro* experiments revealed some of the molecular mechanisms underlying the described neuroprotective effects of cannabinoids in our AD model. ACEA treatment was able to reduce the cytotoxic effect of A $\beta$ <sub>1-42</sub> oligomers in a primary cortical neuron culture in the same way that reversed the amyloid-induced dephosphorylation of protein kinase GSK3 $\beta$ , which is known to play an important role in the pathogenesis of AD.

The comprehensive identification of the pathways by which cannabinoid agonists ameliorated the symptoms and the associated molecular pathology in our experimental model will reinforce the hypothesis that endocannabinoid system could be considered as potential target for the development of novel therapeutic strategies against AD.

**NOTAS:**

**PROLONGED ORAL CANNABINOID ADMINISTRATION PREVENTS NEUROINFLAMMATION AND IMPROVES COGNITIVE DEFICITS IN A TRANSGENIC MODEL OF ALZHEIMER'S DISEASE**

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Cannabinoids are neuroprotective and anti-inflammatory agents with therapeutic potential. Alzheimer's disease (AD) brain shows an ongoing inflammatory condition and non-steroidal anti-inflammatories diminish the risk of suffering the neurologic disease. We have studied the effects of prolonged oral administration of transgenic APP mice with two pharmacologically different cannabinoids (WIN 55,212-2 and JWH-133, 0.2 mg/kg/day in the drinking water during 4 months) on inflammatory and cognitive parameters. The acquisition of a spatial navigation ability was slightly reduced in 9 month old transgenics but it was unaffected by cannabinoid treatment for 2 months. Continuous cannabinoid treatment neither changed memory after additional 2 months and all the animals improved their performance in the water maze, irrespective of genotype or treatment. Novel object recognition was significantly reduced in 11 month old Tg APP mice and 4 month administration of JWH was able to normalize this cognitive deficit. Hippocampal GFAP immunoreactivity and cortical protein expression was unaffected by genotype or treatment. In contrast, the density of Iba1 positive microglia was increased in Tg APP mice, and normalized following JWH continuous treatment. Both cannabinoids were effective at reducing the enhancement of COX-2 and TNF- $\alpha$  expression found in the AD model. Finally, increased cortical  $\beta$ -amyloid levels were significantly reduced in the mouse model by both cannabinoids. We have shown that chronically administered cannabinoid showed marked beneficial effects concomitant with inflammation reduction.

**NOTAS:**

## CHANGES IN SPECIFIC COMPONENTS OF THE ENDOCANNABINOID SYSTEM IN SPINOCEREBELLAR ATAXIAS

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Spinocerebellar ataxias (SCAs) are a group of neurodegenerative diseases, clinically and genetically heterogeneous, characterized by loss of balance and motor incoordination due to dysfunction/degeneration of the cerebellum and its afferent and efferent connections. SCAs belong to the family of disorders caused by expansion of a polyQ tract, so-called polyglutaminopathies. Our group has demonstrated that cannabinoids are effective in the treatment of motor diseases such as Huntington's (which is also caused by CAG triplet repeat expansion) and Parkinson's disease (which presents symptoms similar to some SCAs, e.g. SCA2). In these diseases, cannabinoids improve the motor symptoms and attenuate the progression of brain damage. However, SCAs remain to be studied in relation with a possible therapy with cannabinoids. With this idea in mind, we wanted to study first the status of the endocannabinoid system in this disease using postmortem human brains from patients with different types of ataxias, obtained from two brain banks (Hospital Clinic, Barcelona, Spain, and Netherlands Brain Bank, Amsterdam, The Netherlands). These samples were used for immunohistochemical analysis of CB<sub>1</sub> and CB<sub>2</sub> receptors and the endocannabinoid-degrading enzyme, FAAH. These analyses proven the existence of differences in CB<sub>1</sub> receptor immunostaining in the cerebellum of patients compared with controls, in particular, we found an increase in immunoreactivity for this receptor that was evident in granular and Purkinje layers but also in the dentate nucleus and areas of white matter. We are presently conducting double-staining experiments aimed at identifying the cells where these changes happen. We did not find any changes in immunostaining for CB<sub>2</sub> receptor and FAAH enzyme. The reproduction of SCA pathology in laboratory animals has been difficult to date, but a few murine models have been recently generated using transgenes bearing human mutated forms. We have developed a colony of transgenic mice for SCA2 (available from JAX® Laboratories), one of the most typical and prevalent SCA disorder. These animals were used for recording disease progression, examining functional (e.g. rotarod) and neuropathological (e.g. Nissl staining) markers, in parallel to changes in various components of the endocannabinoid system. We observed that motor incoordination is evident only when animals are 16 month-old. However, neuronal malfunctioning was already evident at earliest ages. For example, we observed a significant reduction of calbindin immunoreactivity in Purkinje cells of these transgenic mice. We are presently studying the status of the cannabinoid system at this presymptomatic age and also at later stages when cerebellar degeneration is evident. In summary, our study demonstrates that the endocannabinoid system, in particular the CB<sub>1</sub> receptor, is significantly altered in the cerebellum of SCA subjects, which supports the idea that the pharmacological management of this system may have therapeutic value in this disease as happens in other neurodegenerative disorders.

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**NOTAS:**

**OLEYLETHANOLAMIDE IS A POWERFUL NEUROPROTECTANT IN DIFFERENT ANIMAL MODELS OF PARKINSONISM**

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The cannabinoid analogue oleylethanolamide (OEA), an agonist of nuclear PPAR- $\alpha$  receptors and antagonist of TRPV1, has been reported to show cytoprotective properties, and previous studies in our lab have shown its partial neuroprotective efficacy against oxidative stress on culture dopamine neurons as well as in vivo after local treatment in parkinsonism. In this study, the potentially neuroprotective efficacy of systemic OEA has been tested in vivo models of Parkinsonism based on MPTP- and 6-OHDA-induced degeneration of substantia nigra dopamine neurons.

In the first study, the potentially neuroprotective efficacy of systemic OEA was tested in a Parkinsonian model based on acute MPTP action over substantia nigra dopamine neurons. In this model, MPTP induces intense inflammation and TH+ down-regulation with limited loss of TH+ neurons. All mice were treated with systemic OEA (0, 0.5, 1, and 5 mg/kg IP) two hours before and after acute MPTP administration (40 mg/kg IP). Animals were sacrificed 48 h and 1 week, and then OX-6 or TH signal in the dorsal striatum and substantia nigra was quantified. The findings revealed that 5 mg/kg OEA (not the other doses) was able to prevent activation of striatal microglial cells, and significantly attenuated the decrease of TH signal in striatal fibers and substantia nigra neurons.

In the second study, Wistar rats were rendered hemiparkinsonian by intrastriatal injections of 6-OHDA (5 $\mu$ g/ $\mu$ L, three sites of injection). These animals were treated two hours before and two hours after surgery with systemic OEA (0, 0.5, 1, and 5 mg/kg IP). Amphetamine- (5 mg/kg IP) and apomorphine-induced (0.5 mg/kg IP) rotation, paw use (cylinder test) and akinesia (locomotion test) were measured one week and one month after surgery. Then animals were sacrificed, followed by striatal and nigral TH immunostaining and western blotting of striatal synaptophysin expression. Also, 48 h after surgery some animals were sacrificed in order to measure short-term oxidative response through HO-1 signal. The results indicated that, in those animals treated with 5 mg/kg OEA (but not the other doses), all functional deficits were significantly reduced ( $p < 0.05$ ), and 6-OHDA-induced reduction of striatal TH+ density and nigral TH+ neurons was significantly and partially antagonised ( $p < 0.05$ ). Besides, synaptophysin expression was not affected and short-term oxidative response in striatum was reduced.

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**NOTAS:**

## **MODULATION OF BRAIN ENDOCANNABINOID SYSTEM DURING PRIMARY DEMYELINATION AND REMYELINATION**

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Multiple sclerosis (MS) is a complex inflammatory demyelinating disease whose etiology remains unknown. Histopathological data indicate that although demyelination may be initiated by autoreactive T cells migrating from the periphery, lymphocyte recruitment and activation can also follow primary oligodendroglial death. Recent data also indicate that demyelination during MS is accompanied by a remyelination process that ultimately fails, leading to the axonal damage and subsequent disability that characterizes this disease. Brain endocannabinoid (eCB) system has been reported to be dysregulated in MS lesions and in models of secondary demyelination, leading to the hypothesis that its pharmacological modulation may be useful for the treatment of this demyelinating disease. Nevertheless, the possible adaptations of brain eCB system during primary demyelination have not been explored. Here we analyzed the gene expression of different proteins involved in endocannabinoid signaling and/or production using a model of primary demyelination which consists in the administration of the copper chelator cuprizone. Adult C57BL6 mice were treated with 0.3% cuprizone in the diet for 3 and 6 weeks (demyelination), or fed with cuprizone for 6 weeks and allowed to recover for 2 weeks (remyelination). Additional groups of mice were treated in parallel with control diet for the same periods of time. Administration of cuprizone induced a time-dependent loss of luxol fast blue staining and an increase in CD11b (+) cells in the corpus callosum, indicative of myelin loss and inflammation, together with reductions in the gene expression of the myelin proteins MBP and MOG. Withdrawal of the toxin for 2 weeks allowed partial recovery of these parameters. These cellular changes were accompanied by upregulated CB<sub>1</sub>, CB<sub>2</sub> and P2X<sub>7</sub> receptors mRNA expression during cuprizone administration and/or withdrawal. These results support a role for eCB system during demyelination/remyelination.

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**NOTAS:**

## **HYPOCRETINS ARE INVOLVED IN THE REINFORCING PROPERTIES OF THE SYNTHETIC CANNABINOID WIN 55,212-2**

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Emerging evidence suggests that the hypocretinerigic (orexinergic) system is involved in addictive behaviour. In this study, we evaluated the role of these hypothalamic neuropeptides in the reinforcing properties of cannabinoids by using behavioural models in mice. The acute administration of the hypocretin receptor-1 antagonist SB334867 (10 mg/kg) after the acquisition of the synthetic cannabinoid WIN 55,212-2 self-administration blocked this behavioural response. WIN 55,212-2 self-administration behaviour was normalized 24h following SB334867 injection. Moreover, the treatment with SB334867 decreased the breaking point achieved in the progressive ratio schedule, suggesting also an involvement of this receptor in the motivation to self-administer WIN 55,212-2. On the other hand, SB334867 injection 30 minutes before each session of WIN 55,212-2 self-administration during the acquisition period diminished the achievement of acquisition criteria of the synthetic cannabinoid. According with these data, the percentage of acquisition of WIN 55,212-2 self-administration was lower in preprohypocretin knockout mice. Therefore, our study establishes an interaction between the reinforcing properties of cannabinoids and the hypocretinerigic system, suggesting a new target for cannabinoid dependence treatment.

**NOTAS:**

## LOSS OF STRIATAL CB<sub>1</sub> CANNABINOID RECEPTORS IS A KEY PATHOGENIC FACTOR IN HUNTINGTON'S DISEASE

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Endocannabinoids act as neuromodulatory and neuroprotective cues by engaging CB<sub>1</sub> cannabinoid receptors. These receptors are highly abundant in the basal ganglia and play a pivotal role in the control of motor behaviour. An early down-regulation of CB<sub>1</sub> receptors has been documented in the basal ganglia of Huntington's disease patients and animal models. However, the pathophysiological impact of this loss of CB<sub>1</sub> receptors in Huntington's disease is as yet unknown. Here, we generated a double-mutant mouse model that expresses human mutant huntingtin exon 1 in a CB<sub>1</sub> receptor-null background, and found that CB<sub>1</sub> receptor deletion aggravates the symptoms, neuropathology and molecular pathology of the disease. Moreover, pharmacological administration of the cannabinoid  $\Delta^9$ -tetrahydrocannabinol to mice expressing human mutant huntingtin exon 1 exerted a therapeutic effect and ameliorated those parameters.

Experiments conducted in striatal cells show that the mutant huntingtin-dependent downregulation of CB<sub>1</sub> receptors involves the control of the CB<sub>1</sub> receptor gene promoter by repressor element-1 silencing transcription factor and sensitizes cells to excitotoxic damage. We also provide in vitro and in vivo evidence supporting that CB<sub>1</sub> receptors control striatal brain-derived neurotrophic factor expression and that the decrease of brain-derived neurotrophic factor levels concomitant to CB<sub>1</sub> receptor loss may contribute significantly to striatal damage in Huntington's disease. Altogether, these results support the notion that down-regulation of CB<sub>1</sub> receptors is a key pathogenic event in Huntington's disease, and suggest that activation of CB<sub>1</sub> receptors in Huntington's disease patients may attenuate disease progression.

**NOTAS:**

**CB<sub>1</sub> CANNABINOID RECEPTOR IN THE DEVELOPMENT AND EXPRESSION OF OSTEOARTHRITIS**

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Osteoarthritis is a degenerative slowly progressing joint disease that affects more than 10 million people worldwide and represents a major healthcare burden. Multiple joint tissues (i.e. subchondral bone, synovium) are involved in the physiopathology of this chronic disease, which is typically associated with the degradation of articular cartilage. While there are considerable insights into the mechanisms underlying tissue remodelling, the precise link between structural tissue changes and the generation of chronic pain experienced by osteoarthritis patients remains unclear. Here, we use two experimental models of osteoarthritis to evaluate the involvement of CB<sub>1</sub> cannabinoid receptors in the development and expression of these pathological manifestations. In these models, we combine the evaluation of both osteoarthritic tissue remodelling and pain related behaviour in mouse, by using a partial medial meniscectomy or sodium monoiodoacetate injection in the knee joint. In the ipsilateral hind paw, a marked and long-lasting mechanical allodynia was observed in both osteoarthritis models, whereas neither thermal hyperalgesia nor thermal allodynia were revealed. The histological evaluation demonstrated structural alterations developed over the course of the next 6 weeks after the induction of the osteoarthritis in both models. Taking into account the crucial role played by the cannabinoid system in other chronic pain states and the existing evidence about cannabinoids as promising agents for the treatment of osteoarthritis, we investigated the involvement of the endocannabinoid system in the modulation of this pathological state. For this purpose, we evaluated the involvement of CB<sub>1</sub> cannabinoid receptor in the behavioural and histological alterations associated to osteoarthritis by using CB<sub>1</sub> knockout mice. Before the induction of osteoarthritis, tactile withdrawal thresholds were similar in both CB<sub>1</sub> knockout mice and their wild-type littermates. After the injection of sodium monoiodoacetate in the knee joint, both genotypes also showed similar levels of mechanical allodynia following the application of tactile stimuli. These results suggest that CB<sub>1</sub> receptor is not mainly implicated in the development and expression of osteoarthritis.

**NOTAS:**

**AUTOPHAGY DURING NEUROENDOCRINE DIFFERENTIATION OF LNCaP CELLS. ROLE OF CANNABINOIDS**

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The growth of the prostate is regulated by humoral factors such as steroids and autocrine/paracrine growth factors. In prostate cancer, neuroendocrine differentiation (NED) is proposed to be a contributing factor of increased aggressiveness and androgen independence of the disease. NE cells produce a variety of neurosecretory products that can regulate cellular growth so that, although the NE cells appear to be nonmitotic, the carcinoma cells adjacent to these NE foci have been noted to exhibit increased proliferative activity. LNCaP is an androgen-responsive prostate cancer cell line that develops a NE phenotype when grown in the absence of serum, androgens or in response to increased intracellular cAMP levels. We analysed NED of LNCaP cells using the microarray approach. We identified up-regulation of lysosome-associated membrane protein type 2 (LAMP-2) along with different neuroendocrine markers such as neuron-specific enolase (NSE). The data obtained from microarray analysis were then validated using quantitative real-time PCR and Western blot analysis. LAMP-2 is related to chaperone-mediated autophagy (CMA), a selective type of autophagy responsible for the lysosomal degradation of soluble cytosolic proteins. These results suggest a role of autophagy in NED of prostate cancer.

In this cellular model of NE differentiated prostate cancer cells, we investigated the effect of the cannabinoid agonists WIN, CB1 and CB2 agonist, and JWH-015, CB2 agonist. First, we tested whether treatment of LNCaP cells with WIN or JWH-015 prevents NED of LNCaP cells. Serum deprived cells were treated from the start of deprivation with increased concentrations of cannabinoids. Doses of 0.5 and 1.5  $\mu\text{M}$  of both cannabinoids allow cells to develop neurites. At 3  $\mu\text{M}$  cells arrest NED whereas higher concentrations kill these cells. We then tested whether these cannabinoids could revert NED observed after 6 days of serum deprivation. We treated NED cells with the same concentrations of the above mentioned cannabinoids for 48 hours. Both, WIN and JWH-015, have no effect on neuroendocrine phenotype at 0.5 and 1.5  $\mu\text{M}$  whereas higher concentrations reverts NED. We also observed an important decrease of the cell number when treated with 5 and 10  $\mu\text{M}$  of WIN but not with JWH-015 treatment. These preliminary results suggest a different role for CB1 and CB2 in NED of LNCaP cells.

**NOTAS:**

## **THE PUTATIVE CANNABINOID RECEPTOR GPR55 PROMOTES CANCER CELL PROLIFERATION VIA ERK**

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GPR55 is an orphan G protein-coupled receptor that may be engaged by some lipid ligands such as lysophosphatidylinositol and cannabinoid-type compounds. Very little is known about its expression pattern and physio-pathological relevance, and its pharmacology and signaling are still rather controversial.

Here we analyzed the expression and function of GPR55 in cancer cells. Our data show that GPR55 expression in human tumors from different origins correlates with their aggressiveness. Moreover, GPR55 promotes cancer cell proliferation, both in cell cultures and in xenografted mice, through the overactivation of the extracellular signal-regulated kinase cascade (ERK cascade).

These findings reveal the importance of GPR55 in human cancer, and suggest that it could constitute a new biomarker and therapeutic target in oncology.

**NOTAS:**

## SIMULTANEOUS SYMPTOM-RELIEVING AND NEUROPROTECTIVE TREATMENT WITH $\Delta^9$ -THCV IN ANIMAL MODELS OF PARKINSON'S DISEASE

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The phytocannabinoids,  $\Delta^9$ -THC and CBD, protect nigral neurons in rat models of Parkinson's disease (PD). This effect is independent of cannabinoid receptors but related to the antioxidant properties of these compounds, although an additional CB<sub>2</sub> receptor-mediated modulation of glial influences on neurons cannot be ruled out. On the other hand, blockade of CB<sub>1</sub> receptors has been reported to alleviate motor inhibition in parkinsonian rats. These previous findings strongly support the hypothesis that a cannabinoid having antioxidant properties and the ability to activate CB<sub>2</sub> receptors, but to block CB<sub>1</sub> receptors, might be a promising therapy both for alleviating parkinsonian symptoms and for delaying neurodegeneration in PD. Such pharmacological effects can be induced by the phytocannabinoid  $\Delta^9$ -THCV, which has been investigated in rats receiving an i.c.v. injection of 6-hydroxydopamine, a model that may be used to monitor simultaneously parkinsonian symptoms and signs of neuroprotection. In these animals, acute administration of  $\Delta^9$ -THCV (2 mg/kg, i.p.) attenuated the motor inhibition caused by 6-hydroxydopamine with the same potency as rimonabant. This effect seemed to result from  $\Delta^9$ -THCV-induced changes in glutamatergic transmission. Also in these animals, chronic administration of  $\Delta^9$ -THCV (2 mg/kg, i.p.; 14 days) partially attenuated the damage caused to the substantia nigra, as indicated by tyrosine hydroxylase and OX-42 immunostaining. We assume that neuroprotective effects of  $\Delta^9$ -THCV in 6-hydroxydopamine-lesioned rats are likely due to its antioxidant properties rather than to its ability to activate CB<sub>2</sub> receptors, because neuroprotection was also attained, to an even greater extent, with CBD-enriched botanical extract. In concordance with this idea, CB<sub>2</sub> receptor-deficient mice responded to 6-hydroxydopamine lesions to a similar extent as wild-type animals, whereas CB<sub>2</sub> receptors were poorly up-regulated in the rat substantia nigra in response to 6-hydroxydopamine. This was not the case in the inflammatory model of PD in which mice receive an intrastriatal injection of LPS. The substantia nigra of these animals exhibited a greater up-regulation of CB<sub>2</sub> receptors in response to LPS, and, in these animals, administration of  $\Delta^9$ -THCV (2 mg/kg, i.p.) was associated with a complete preservation of tyrosine hydroxylase-positive neurons, an effect that presumably involved CB<sub>2</sub> receptors. In summary, given its likely antioxidant properties and its ability to activate CB<sub>2</sub> but to block CB<sub>1</sub> receptors,  $\Delta^9$ -THCV seems to have a promising pharmacological profile both for delaying disease progression in PD, and also, in contrast to CBD and  $\Delta^9$ -THC, for ameliorating parkinsonian symptoms.

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**NOTAS:**

## **THE ACTIVATION OF THE ENDOCANNABINOID SYSTEM REDUCES THE TAT INDUCED-EXPRESSION OF PRO-INFLAMMATORY PARAMETERS IN GLIAL CELLS**

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The HIV regulatory protein Tat is a potent transactivator of viral and cellular gene expression as well as an important mediator of neurotoxicity. Tat may act directly on neurons causing excitotoxicity and cell death by apoptosis but also indirectly stimulating the production and release of pro-inflammatory and neurotoxic mediators by glial cells and macrophages. In the last few years, numerous *in vivo* and *in vitro* experiments propose the activation of the endocannabinoid system (ECS) as an alternative neuroprotective therapeutic approach that has the potential to be devoid of undesirable side effects for a long-term use. In the present work, we study whether the potentiation of the endogenous cannabinoid tone exerts beneficial effects by blocking the expression of inflammatory mediators in the response triggered by Tat in glial cells.

To that end, primary culture of mixed glial cells (astrocytes and microglia) from wild type and knock out FAAH mice were transfected with the expression plasmid CMV-Tat during 48 hours. The corresponding empty expression vector (pCMV-500) was used as negative control. Then, we analyzed the changes in the mRNA expression of some of the most relevant inflammatory cytokines and enzymes. We observed that the deletion of the enzyme FAAH produced a statistically significant decrease in iNOS, TNF- $\alpha$  and IL-1 $\beta$  mRNA levels with respect to wild type cells. Our results seem to confirm that the ECS activation also produced beneficial effects in the inflammatory response triggered by stimulated glial cells.

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**NOTAS:**

**ROLE OF mTOR IN THE PHARMACOLOGICAL EFFECTS OF THC**

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The potential therapeutic benefits of certain cannabinoid compounds have raised interest in understanding the molecular mechanisms that underlie some of the cannabinoid-mediated effects. We previously demonstrated that the mammalian target of rapamycin (mTOR), which is a protein kinase highly involved in the regulation of protein translation and synaptic plasticity, was responsible of the memory impairment produced by  $\Delta^9$ -tetrahydrocannabinol (THC). In the present study, we found that the activation of the mTOR pathway is also required for the anxiogenic-like response produced by a high dose of THC (10 mg/kg, ip) since mTOR inhibition blocked this effect. In contrast, mTOR is not involved in the anxiolytic-like response produced by a low dose of THC (0.3 mg/kg). Moreover, THC-induced hypolocomotion and antinociception were also independent of mTOR activation. Therefore, mTOR inhibition preserved the therapeutic benefits of THC, such as the anxiolytic or antinociceptive effects, and prevented the negative effects, such as the anxiogenic and amnesic-like responses. On the other hand, a clear tolerance to the hypothermic, hypolocomotor and antinociceptive effect of THC was observed after a chronic treatment (10 mg/kg, 6 days). Interestingly, although the CB1 cannabinoid receptor was dramatically downregulated, no tolerance to the THC-induced anxiogenic-like effect and memory impairment was developed. In addition, after the cessation of THC treatment, mice required around 5 days to recover the cognitive impairment. This effect was associated to mTOR activation in the hippocampus after the chronic treatment and during the recovery memory period, and it was also blocked by mTOR inhibition. Altogether, these results provide new insights to better target the endocannabinoid system for therapeutic purposes.

**NOTAS:**

## COMPARATIVE STUDY OF DIFFERENT VEHICLES ON THE CANNABINOID TETRAD IN MICE

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Cannabinoids are compounds with a lot of potential therapeutic applications, but one of the problems of these compounds is their poor solubility. So we asked ourselves: does the vehicle, in which they are dissolved, modify their effects?. There is not any comparative study on cannabinoid solvents or the effectiveness of cannabinoids according to the vehicle in which they are dissolved; this may hinder the comparison of results. So, our first aim was to evaluate the influence of different vehicles, that are frequently used in the dissolution of cannabinoids, on the well known cannabinoid tetrad in mice; and then, to compare the effect of WIN 55,212 (WIN) dissolved in these different vehicles. Vehicles used were: Tween 80/ethanol/saline<sup>1</sup>, Tocrisolve, and Dimethylsulphoxide (DMSO) at different concentrations (10, 20, 25, and 50%).

ICR male mice (25-30 g) were used. To evaluate the activity of the vehicles and WIN (2.5 mg/kg) nociception, body temperature, catalepsy and locomotor activity were studied on the cannabinoid tetrad where cannabinoids manifest antinociceptive effects, hypothermia, catalepsy and decreased locomotor activity<sup>2</sup>. For this, we measured body temperature through rectal probe insertion, nociception with a hot-plate at 55 °C, catalepsy using a modified “ring test” originally described by Pertwee<sup>2</sup>, and the motor activity was evaluated with a rota-rod apparatus. All vehicles and WIN were administered intraperitoneally and the effect was studied 30 min after their administration.

All the vehicles tested behaved similarly on the cannabinoid tetrad; so, none of them produced antinociception, hypothermia, catalepsy nor alterations on locomotor activity, except DMSO 50 % that modified all signs of the tetrad ( $p < 0.001$ ) and DMSO 25% that reduced rectal temperature ( $p < 0.05$ ). The effect of WIN dissolved in Tween 80/ethanol/saline, Tocrisolve or DMSO 20% was similar; it produced antinociception, hypothermia, catalepsy and reduced locomotion.

Our data suggest that Tween 80/ethanol/saline, Tocrisolve and DMSO 20% are good vehicles to use on the cannabinoid tetrad. DMSO at a concentration greater than 20% can not be used because it alters the results. WIN 55,212 can be dissolved in any of these vehicles because it produces the same effect on the mouse tetrad assay.

<sup>1</sup> Pertwee, *et al*, 1992. *Br. J. Pharmacol.*, 105 (4): 980–984.

<sup>2</sup> Pertwee *et al*, 1972. *Br. J. Pharmacol.*, 46 (4): 753–763.

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**NOTAS:**

## HYDROSOLUBLE CANNABINOID COMPOUNDS: SYNTHESIS, BIOLOGICAL EVALUATION AND METABOLISM STUDIES

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Metabolic disfunctions such as type 2 diabetes, cardiovascular diseases and cancer associated with obesity are now becoming a massive clinical and public health challenge. The therapies included sibutramine, a noradrenaline and serotonin reuptake inhibitor drug and rimonabant, a cannabinoid receptor antagonist. Both were removed from the market due to adverse effects. Orlistat, a gastrointestinal lipase inhibitor, is the only currently marketed anti-obesity drug with limited prescription due to undesirable side effects.

Cannabinoid CB1 receptor antagonists have proved to be efficient for treating obesity and associated metabolic disorders. However rimonabant, first in this class therapy, has shown to induce depressed mood disorders. Since cannabinoid CB1 receptors are highly abundant in the brain, they mediate these adverse effects limiting the therapeutic value of such drug. CB1 receptors are also present in peripheral tissues involved in metabolic control including endocrine pancreas, skeletal muscle and liver. Thus, peripheral CB1 receptors represent an important target by which CB1 antagonists regulate energy balance. Therefore, there is a real need for developing a peripherally restricted CB1 receptors antagonist.<sup>1</sup>

Our approach to the design of novel CB1 antagonists focused on conjugating a poly(ethylene glycol) (PEG) polymer to the pyrazole scaffold of rimonabant. This polymer is nontoxic, soluble in water and FDA approved.<sup>2</sup> Based on this strategy, a series of PEGylated pyrazole derivatives was synthesized. Unlike most of the cannabinoid ligands they are water-soluble. In the present work, the synthesis, the pharmacological evaluation and the metabolism studies of these new compounds are reported.

### References:

- 1) D. R. Janero, A. Makriyannis. *Cannabinoid receptor antagonists: pharmacological opportunities, clinical experience, and translational prognosis*. Expert Opin. Emerg. Drugs, 2009; 14: 43-65.
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**NOTAS:**

## THE INVERSE AGONIST EFFECT OF RIMONABANT IN HUMAN POSTMORTEM BRAIN IS MEDIATED BY $G_{\alpha i3}/G_{\alpha o}$ PROTEINS

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**Introduction:** Several evidences suggest that rimonabant behaves as an inverse agonist in different biological systems, in both *in vitro* and *in vivo* assays. Previous studies of our research group have shown that this inverse agonist effect might not be mediated by the cannabinoid CB<sub>1</sub> receptor. However, the specific mechanism underlying these inverse agonist properties is still known.

**Aim:** The aim of this study was to further characterize the inhibitory effects of rimonabant on G-protein activation in postmortem human brain membranes using the [<sup>35</sup>S]GTPγS scintillation proximity assays (SPA) combined with the use of specific antibody-capture of the different G<sub>α</sub> subunits.

### *Material and Methods:*

- 1) [<sup>35</sup>S]GTPγS binding assays in human brain cortical membranes were performed with a unique concentration of the cannabinoid ligands rimonabant and/or WIN55,212-2 (10<sup>-5</sup> M).
- 2) After [<sup>35</sup>S]GTPγS binding membranes were solubilised and incubated in the presence of specific antibodies developed against the different G protein alpha subunits (G<sub>αi1</sub>, G<sub>αi2</sub>, G<sub>αi3/o</sub>, G<sub>αo</sub>, G<sub>αq/11</sub> and G<sub>αs</sub>).
- 3) At last PVT SPA beads coupled with secondary antibodies were added allowing the detection of the radioligand bound to each of the specific G<sub>α</sub> subunits.

### *Results:*

1) WIN55,212-2 (10<sup>-5</sup> M) induced a statistically significant stimulation of the [<sup>35</sup>S]GTPγS binding mediated by G<sub>αi1</sub> (191±17%, p=0.006), G<sub>αi2</sub> (135±11%, p=0.046) and G<sub>αo</sub> (159±9%, p=0.003), but not by G<sub>αq/11</sub> (101±6%, p=0.874) nor G<sub>αs</sub> (95±3%, p=0.179). The low selectivity of the anti-G<sub>αi3/o</sub> antibody did not allow us to discriminate which part of the stimulation observed (220±17%, p=0.002) corresponds to each of the G-protein subtypes. The WIN 55,212-2 stimulation was always reverted to basal binding values by the co-incubation with rimonabant 10<sup>-5</sup> M with the exception of G<sub>αi1</sub> (158±7%, p=0.0001).

2) The incubation in the presence of rimonabant 10<sup>-5</sup> M induced a statistically significant stimulation over the basal binding value when the anti-G<sub>αi1</sub> specific antibody was used (128±4%, p=0.006). On the other hand, a reduction of the basal [<sup>35</sup>S]GTPγS binding values was observed when both anti-G<sub>αi3/o</sub> (86±4%, p=0.021) or anti-G<sub>αo</sub> (86±3%, p=0.007) antibodies were used.

**Conclusion:** These data suggest that the apparent inverse agonist effect of rimonabant over G-protein activity in human brain membranes is mainly exerted by its action over G<sub>αi3/o</sub> subunits. Moreover, rimonabant seems to display a partial agonist-like effect through the G<sub>αi1</sub> protein.

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**NOTAS:**

**THE SPECIFIC CB<sub>2</sub> AGONIST, HU-910, DOES NOT MODIFY DISEASE PROGRESSION IN AN ANIMAL MODEL OF ALZHEIMER'S DISEASE**

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The endocannabinoid system participates in the response of the CNS against acute and chronic insults and its modulation has been postulated as a therapeutic approach for the treatment of several diseases. Among other aspects, the induction of cannabinoid CB<sub>2</sub> receptors and the possible influence of cannabinoid ligands, on the inflammation triggered by the beta amyloid peptide (A $\beta$ ) have attracted considerable attention. To analyze these issues, we used the 5xFAD-mouse model of amyloid deposition 6 and 9 months-old mice were treated daily i.p. with the potent and selective CB<sub>2</sub> agonist HU-910 (5mg/kg) for 12 days. Effects of HU-910 on recent memory were analyzed with the water morris maze test. Finally, after sacrifice brains were quickly frozen and the expression of several markers of inflammation was quantified by qRT-PCR.

Our results show that HU-910 does not induce significative changes in recent memory in 5xFAD mice. In addition, no improvement in the expression of inflammatory mediators was observed. Thus, we conclude that, the activation of CB<sub>2</sub> receptor by HU 910 does not produce beneficial effects in this experimental model at any age considered.

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**NOTAS:**

## PRECISE SUBCELLULAR LOCALIZATION OF THE CB1 RECEPTOR IN HIPPOCAMPAL GLIA

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Glial cells are the most abundant cell population in the central nervous system. There are several cell types and it is well established that CB1 receptors are present in astrocytes where they exert relevant physiological roles (for review: Stella, 2010). However, the localization of CB1 in specific glial compartments has remained elusive greatly due to tissue processing conditions and the CB1 antibodies used.

In this study, we have used mice (kindly provided by Dr. Giovanni Marsicano) transcardially perfusion-fixed with Zamboni's fixative (a mixture containing 2% formaldehyde and 15% picric acid in 0.1M phosphate buffer, pH 7.4). Then, brains were removed and hippocampal sections were incubated with rabbit CB1 polyclonal antibody (1:2000, kindly supplied by Dr. Ken Mackie). The tissue was processed for a high resolution pre-embedding immunogold method for electron microscopy. Under these particular conditions, in addition to abundant silver-intensified gold particles decorating many synaptic terminals, accumulation of immunoparticles was also observed in glial portions in the neuropil distributed around synaptic and non-synaptic neuronal compartments in the hippocampal CA1 region.

The subcellular localization of CB1 in glia by using particular tissue conditions for immunocytochemistry as shown here, paves the way for a better understanding of how CB1 in identified astrocytic compartments affects these cells' function in health and disease.

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**NOTAS:**

## **CANNABIDIOL PROTECTS OLIGODENDROCYTE PROGENITORS BY DIRECT AND INDIRECT MECHANISMS: IMMUNOMODULATORY ACTIONS IN A VIRAL MODEL OF MULTIPLE SCLEROSIS**

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Multiple Sclerosis (MS) is an inflammatory demyelinating and neurodegenerative disease of the Central Nervous System, and the most common neurological disease in young adults in the Western World. Following advances in the understanding of the immunological mechanisms that underlie the pathogenesis of MS, cannabinoids has arisen as one of the possible agents with therapeutic potential, as immune cells and neuronal tissues express receptors for these compounds.

In the present study, we have investigated the effect of Cannabidiol (CBD), a non psychoactive component of *Cannabis sativa*, in primary cell cultures of oligodendrocyte progenitor cells (OPCs). Our results show that CBD (1 $\mu$ M) can directly protect OPCs from oxidative stress cell death through mechanisms that involve a decrease in the production of reactive oxygen species. CBD also protects OPCs from direct LPS/IFN $\gamma$  –induced cell death. Moreover, Microglial Conditioned Media from resting or activated microglia treated with CBD can indirectly protect OPCs from inflammatory damage.

The effect of CBD in a viral model of MS (Theiler's virus induced demyelinating disease) show that there is a therapeutic time window (presymptomatic phase) for CBD treatment that improves neurological deficits. These benefits were associated to decreased gene expression of proinflammatory cytokines as well as reduced microglia activation in the spinal cord of TMEV-infected mice.

Further experiments are in progress in order to identify the intracellular pathways and the mechanisms involved in the protective and immunomodulatory actions of CBD.

**NOTAS:**

**NON- PSYCHOACTIVE CANNABINOIDS ATTENUATE MCP-1 EXPRESSION: IMPLICATIONS IN MULTIPLE SCLEROSIS**

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The monocyte chemoattract protein-1/CCL2 (13 KDa) is a member of the CC chemokine family that acts like a potent chemotactic factor for monocytes. The interaction with its receptor, CCR2, has been related to the pathogenesis of Multiple Sclerosis (MS). High levels of MCP-1/CCL2 in CSF and actively demyelinating lesions have been detected in patients with MS. Moreover, CCL2-deficient mice have been found to be markedly resistant to the induction of EAE, and the treatments with anti-CCL3 antibodies were efficacious in inhibiting EAE onset. Several studies support the potential therapeutic value of cannabinoids (CBs) in the treatment of MS and its experimental models but advance will be made in reducing CBs side effects, including unwanted psychoactivity. Then, studies with non-psychoactive cannabinoids are needed. Our current research is focusing on the study of cellular and molecular mechanisms involved in the inhibitory effect of CBs in leukocyte entry into the brain. Previous work from the lab has shown that CBs downregulate adhesion molecule expression in Theiler murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD). Because of the importance of chemokines in leukocyte infiltration we investigated *in vivo* expression of MCP-1/CCL2, MIP-1 $\alpha$ /CCL3 and RANTES/CCL5 during TMEV-IDD development at earlier stages (3 and 7 days post-infection). Our next step was to analyze the effects of CBD and PEA in astrocytes stimulated with IL-1 $\beta$ /TNF- $\alpha$ . Preliminary results showed that CBD and PEA decreases MCP-1 expression at 6 hours. The pharmacological routes confirm that neither CB<sub>1</sub> nor CB<sub>2</sub> are implicated in their effects. These results support the interest of using non-psychoactive cannabinoids as potential therapeutic agents in MS.

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**NOTAS:**

**LONG TERM BENEFICIAL EFFECT OF CANNABIDIOL ON NEUROLOGICAL FUNCTION AFTER NEWBORN HYPOXIA-ISCHEMIA**

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*Aims and scope:* Cannabidiol (CBD) has demonstrated to reduce immature brain damage after hypoxic-ischemic (HI) insults, improving functional neurological recovery. This effect, however, has been demonstrated only in the short term so far. The aim of the present study was to demonstrate that the improvement in functional recovery after HI induced by CBD in immature brain is sustained in the long term. *Methods:* 7-to-10 day-old Wistar rats were exposed to 120 min hypoxia (10% FiO<sub>2</sub>) after left carotid artery electrocoagulation, to induce unilateral (left) HI brain damage. After recovery from hypoxia, pups were treated with a single dose of CBD (1 mg/kg s.c., HC, n= 19) or vehicle (HV, n= 16). Sham-operated pups served as controls, being similarly treated with vehicle (SV, n=11) or CBD (SC, n= 11). Four to five weeks later, rats underwent a neurobehavioral study, assessing the failure in right side motor performance (% of right deficit on beam walking as well as on standing up in a glass cylinder), in coordination (time on rota-rod, in sec) and in memory (% preference to new objects). *Results:* HI induced long term neurological deficit, as demonstrated by the increase in the incidence of right deficit (46.7 and 23.1% on beam walking and cylinder, respect., in HV vs 18% and 10% in SV, p<0.05), and the poorer results in rota-rod (189±22 vs 243±27 sec for HV and SV, respect., p<0.05) and memory (51±9 vs 72±10% preference in HV and SV, respect., p<0.05). CBD reduced the incidence of right deficit (35 and 12% on beam walking and cylinder, respect.), and improved the results in rota-rod (211±22 sec) and memory (67±7% preference). Such beneficial effects tended to be stronger in males. Neurobehavioral tests were similar in SV than in SC. *Conclusions:* post HI administration of CBD to newborn rats improves functional neurological recovery in the long term and is free from side effects on neurological development.

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**NOTAS:**

## THE LOSS OF HINT1 REVEALS THE NMDAR-SENSITIVE COMPONENT OF CANNABINOID INDUCED ANALGESIA

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HINT1 is a member of the histidine triad (HIT) protein superfamily of nucleotide hydrolases and transferases. The HINT1 dimer contains a cluster of zinc-binding residues that can bind other proteins, as well as possessing a strong negative charge. Indeed, it is thought HINT1 might act as a scaffold in G protein coupled receptor signalling. Accordingly, we have shown that HINT1 is essential to maintain the interaction between *N*-methyl-D-aspartate receptors (NMDARs) and the mu opioid receptor in the mouse brain (1). Since NMDARs are also involved in mediating cannabinoid-induced analgesia, we have examined the possible role of HINT1 in the molecular processes underlying the association between the CB1R and NMDAR. In a mouse 129SvJ strain carrying a targeted disruption of HINT1, intracerebroventricular (icv) administration of the cannabinoid agonists WIN 55,212-2 and methanandamide produced a dose-dependent analgesic response similar to that in WT mice. Similarly, the development of acute tolerance did not differ in these knockout or WT animals. However, pretreatment with dizocilpine (MK-801), a competitive NMDA receptor antagonist, was capable of blocking the cannabinoid induced analgesia in WT mice but not in KO mice. Moreover, in the absence of HINT1 the CB1R became supersensitive, and a profound and lasting desensitization of this receptor developed. We conclude that HINT1 is essential to preserve the regulation exerted by the NMDA receptor on the analgesia induced by cannabinoids.

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1 Rodríguez-Muñoz M, de la Torre-Madrid E, Sánchez-Blázquez P, Wang JB and Garzón J, (2008). *Cell Signal* **20**:1855-1864

2 Thorat SN and Bhargava HN, (1994). *Brain Res* **667**:77-82

**NOTAS:**

## URB602, A MONOACYLGLYCEROL LIPASE INHIBITOR, PROTECTS FROM THE LONG-TERM CONSEQUENCES OF A NEONATAL HYPOXIC-ISCHEMIC BRAIN INJURY IN RATS

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**Background and purpose:** In recent years, increased interest has been given to the neuroprotective properties of endogenous cannabinoids (endocannabinoids), that bind to the same cannabinoid receptors (CB1, CB2) that mediate the effect of  $\Delta^9$ -tetrahydrocannabinol, the active compound of cannabis. *N*-arachidonoyl ethanolamine (anandamide; AEA) and 2-arachidonoylglycerol (2-AG) are the most important endocannabinoids. After brain injury the level of AEA and 2-AG are increased (3) and administration of cannabinoid agonists can reduce injury, both *in vitro* (1,2) and *in vivo* (4,5). We investigated whether increasing the levels of 2-AG or AEA by inhibition of the monoacylglycerol lipase (MGL) or the fatty acid amide hydrolase (FAAH), the enzymes that deactivates the endocannabinoids 2-AG and AEA, respectively, can provide long-lasting neuroprotective effects in a neonatal model of hypoxia-ischemia (HI).

**Methods:** On postnatal-day (PN) 7, newborn rats were subjected to permanent ligation of the right common carotid artery followed by 2.5 h hypoxia. The MGL inhibitor URB602 or the FAAH inhibitor URB597 (25  $\mu$ g in 4  $\mu$ l of 35% DMSO) were administered 30 min before of HI. The neuroprotective effect was evaluated by assessing necrotic (propidium iodide, PI) and apoptotic (caspase-3 expression) cell death in animals sacrificed 24 h after HI, by histological methods at PN14 and adulthood, and by using behavioural tests (T-maze and circular water maze).

**Results:** URB602 administration significantly reduced the increased apoptotic and necrotic cell death observed 24 h after HI. At adulthood, URB602-treated HI animals performed better both the T-maze and the circular water maze, and showed significant reduction of the brain damage. URB597, in contrast, did not reduce brain injury.

**Conclusion:** These results indicate that inhibition of MGL by administration of URB602 significantly reduces brain damage and improves functional outcomes in neonatal HI. The reduced activation of caspase-3 and PI labeling suggests that the neuroprotective effect may be related to a dampening of both apoptotic and necrotic cell death.

1. Nagayama et al., 1999. 19: 2987-2995
2. Sinor et al., 2000. 278: 157-160
3. Panikashvili et al., 2001. 413: 527-531
4. Martinez-Orgado et al., 2003. 114:132-139

5. Fernandez-Lopez et al., 2007. 62: 225-260

**NOTAS:**

## NEUROPROTECTIVE EFFECTS OF PHYTOCANNABINOID-BASED MEDICINES IN A RAT MODEL OF HUNTINGTON'S DISEASE

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Several cannabinoid agonists afford neuroprotection in experimental models of Huntington's disease (HD). In this study, we examined whether combinations of botanical extracts enriched with either  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) or cannabidiol (CBD), which are the main constituents of the cannabis-based medicine Sativex<sup>®</sup>, may also provide neuroprotection in the rat model of HD that relies on 3-nitropropionate intoxication. Although this neurotoxin was systemically administered, it produced selective damage in the striatum as indicated by GABA depletion, a reduction in the number of Nissl-stained neurons and a low expression of the antioxidant enzyme superoxide dismutase-1 (SOD-1) and the neurotrophin IGF-1. In addition, 3NP intoxication was followed by up-regulation of the calcium-binding protein calpain. The administration of  $\Delta^9$ -THC- and CBD-enriched botanical extracts combined in a  $\Delta^9$ -THC:CBD ratio of 1:1 as in Sativex<sup>®</sup> (total cannabinoid administered equivalent to 3 mg/kg of both phytocannabinoids) attenuated 3NP-induced GABA depletion, loss of Nissl-stained neurons and up-regulation of calpain, whereas it completely reversed the reduction in SOD-1 and IGF-1 expression. Similar responses were generally found with other combinations of  $\Delta^9$ -THC- and CBD-enriched botanical extracts (i.e. 2:1 and 1:2; total cannabinoid administered equivalent to 3 mg/kg of both phytocannabinoids), thus indirectly suggesting that these effects are probably related more to the antioxidant and cannabinoid receptor-independent properties of both phytocannabinoids than to their activation of CB<sub>1</sub> and/or CB<sub>2</sub> receptors. In fact, selective antagonists for each of these receptor types, i.e. SR141716 and AM630, respectively, were unable to prevent the positive effects on calpain expression caused in 3NP-intoxicated rats by the 1:1 combination of  $\Delta^9$ -THC and CBD. In summary, this study provides preclinical evidence in support of a beneficial effect of the cannabis-based medicine Sativex<sup>®</sup> as a neuroprotective agent capable of delaying disease progression in HD, a disorder that is currently poorly managed in the clinic, prompting an urgent need for clinical trials with agents showing positive results in preclinical studies.

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**NOTAS:**

## REGULATION OF SOMATODENDRITIC 5-HT<sub>1A</sub> RECEPTORS BY URB597 ALONE OR IN COMBINATION WITH THE ANTIDEPRESSANT FLUOXETINE IN MICE

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Previous reports indicate that endocannabinoid system may be involved in the pathogenesis of depression probably through its interaction with the serotonergic system in the brain. In recent reports it has been described that FAAH (fatty acid amine hidrolase) inhibitors exhibit antidepressant like-effects in some acute tests predictive of antidepressant efficacy. It has been also proposed that the therapeutic effects of antidepressant drugs might be related to adaptive changes in serotonergic neurotransmission, through the activation of the different serotonin receptor subtypes, including 5-HT<sub>1A</sub> receptors.

To extend our knowledge on the crosstalk mechanisms between brain endocannabinoid and serotonergic systems and their implication in depression, we have evaluated the influence of chronic administration of a FAAH inhibitor alone or in combination with the antidepressant fluoxetine in the functionality of 5-HT<sub>1A</sub> receptors in the brain by using *in vitro* and *in vivo* experimental procedures.

Male mice were treated with vehicle (1% DMSO i.p.), fluoxetine (160 mg/l p.o.), URB597 (0.3 mg/kg i.p.) and their combination during 3, 7, 14, 21 and 28 days. After 28 days of treatment, 8-OH-DPAT-induced stimulation of [<sup>35</sup>S]GTPγS binding in the dorsal raphe nucleus (DRN) was attenuated by chronic fluoxetine (% red= 62.3; p<0.01 ) and URB597 (% red= 35.6 ; p<0.05 as well as their combination (% red= 85.6; p<0.01). In order to confirm whether the desensitization of 5-HT<sub>1A</sub> receptors at the level of G-protein coupling has a functional relevance in DRN, we carried out *in vivo* experiments at different periods of treatment. In vehicle-treated group 8-OH-DPAT- induced a significant decrease of body temperature (-2.6°C ± 0.1°C,) with a maximal effect 20 minutes post-injection. Chronic treatment with fluoxetine decreased significantly (p< 0.01 vs vehicle-treated mice) the hypothermic effect of 8-OH-DPAT from the third day of treatment until the end; while 7 days treatment were required for the URB597 to significantly desensitize 5-HT<sub>1A</sub> autoreceptors (p< 0.05 vs vehicle), this effect lacking statistical significance after 28 days of treatment. The combination of both drugs showed similar effects to those induced by fluoxetine alone.

In conclusion, our data indicate that chronic URB597 induces some neurochemical adaptive changes in 5-HT<sub>1A</sub> autoreceptors similar to those found with the classical SSRI fluoxetine. However the combination of the FAAH inhibitor with the antidepressant does not result in a potentiation of the response, at least in this experimental approach.

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**NOTAS:**

**EXPRESSION OF THE mRNAs CODING THE CANNABINOID RECEPTORS 1 AND 2 IN THE PALLIDAL COMPLEX OF MACACA FASCICULARIS**

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The putative presence of the endocannabinoid receptor type 2 (CB2-R) in the central nervous systems is still a matter of debate. Although firstly described in peripheral and immune tissues, evidences suggesting the existence of CB2-Rs in glial cells and even neurons have been made available more recently. By taking advantage of newly-designed CB2-R mRNAs riboprobes, we have demonstrated by in situ hybridization (ISH) and PCR the existence of CB2-R transcripts in a variety of brain areas of the primate *Macaca fascicularis*, including the cerebral cortex, the hippocampus as well as in the external and internal divisions of the globus pallidus (GPe and GPi, respectively), both pallidal segments showing the highest abundance of CB2-R transcripts. Furthermore, in the second part of the study, basal ganglia output neurons (pallidothalamic projection neurons) were identified following the deposit of the retrograde tracer cholera toxin (CTB) in the primate ventral thalamic nuclei. Next, the presence of CB1 and/or CB2 transcripts within CTB+ neurons was assessed by combining the immunofluorescent detection of CTB together with dual fluorescent ISH using riboprobes directed to the mRNAs coding CB1 and CB2 receptors. Obtained results showed that (i) pallidothalamic-projecting neurons contain transcripts coding for CB1 and CB2 receptors, (ii) CB1 and CB2 transcripts share an identical subcellular distribution within the cytoplasm of basal ganglia output neurons, and (iii) when compared to the expression levels obtained in control primates, there is a marked downregulation of CB1 and CB2 gene expression levels in MPTP-treated monkeys, together with an upregulation of CB1 and CB2 mRNA expression levels when considering dyskinetic primates. These data paves the way for the potential choice of endocannabinoid receptors as promising pharmacological targets for the treatment of movement disorders.

**NOTAS:**

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