UNIVERSITI PUTRA MALAYSIA

CB1-MEDIATED EFFECTS OF DELTA-9-TETRAHYDROCANNABINOL (Δ9THC) ON NEURONAL PROTEIN EXPRESSIONS IN THE HIPPOCAMPUS OF MALE *SPRAGUE DAWLEY* RATS

FATIN NADZIRAH BINTI ZAKARIA

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By

FATIN NADZIRAH BINTI ZAKARIA

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

CB_1-MEDIATED EFFECTS OF DELTA-9-TETRAHYDROCANNABINOL (Δ^9THC) ON NEURONAL PROTEIN EXPRESSIONS IN THE HIPPOCAMPUS OF MALE *SPRAGUE DAWLEY* RATS

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Chairman : Mohamad Aris Bin Mohd Moklas, PhD

Faculty : Medicine and Health Sciences

Neuroplasticity refers to the ability of the brain to respond as a result of a certain experience. Delta-9-tetrahydrocannabinol (Δ^9THC), originally extracted from the female plant of *Cannabis sativa* is regarded as the most active psychotropic ingredient of cannabis. *Cannabis sativa*, the largest variety grows in both tropical and temperate climates. Δ^9THC has been shown to affect sensory perception, cognition and memory, reward, appetite, motor coordination and attention. A recent review of references indicates that Δ^9THC plays an important role in neuronal plasticity. *In-vitro* and *in-vivo* studies showed that administration of Δ^9THC modulates several neuronal protein expressions involved in synaptic plasticity in hippocampal region. Previous studies have provided clues on how hippocampus plays an important role in memory process. However the molecular alteration and cellular mechanism leading to neuronal plasticity

are not yet well understood. Therefore, the objectives of this study were to evaluate the effects of acute and chronic Δ^9THC treatment on total ERK1 and ERK2 (p44 and p42 MAP kinase), p-ERK1 and p-ERK2 (p-p44 and p-p42 MAP kinase), CREB, p-CREB and *c-fos* protein levels in the rat hippocampus of brain by studying the CB_1 receptor mechanism which includes the receptor agonist and antagonist. These proteins are thought to be involved in neuronal plasticity. This study was done in 3 experiments. Experiment 1 and 2, the rats were divided into 4 groups which were control group and three treated groups (0.5, 1.0 and 2.0 mg/kg Δ^9THC) respectively. For acute treatment (experiment 1), the rats in control group received vehicle (2% ethanol + 0.9% NaCl) only while for treated group, the rats received 0.5, 1.0 and 2.0 mg/kg of Δ^9THC which were administered every 48 hours for 7 days. For chronic treatment (experiment 2), Δ^9THC (0.5, 1.0 and 2.0 mg/kg) were administered every 48 hours for 21 days. All drugs and vehicle were administered via intraperitoneal injection (i.p.). The volume of i.p. injection was 0.1 ml/100 g body weight. Based on the chronic study, 2.0 mg/kg Δ^9THC which was the optimum dose were used in the combination treatment (experiment 3) to study the effect of chronic Δ^9THC with pretreatment CB_1 antagonist SR141716A (rimonabant). All drugs solution was prepared immediately prior to the experiment. The data were corrected on the basis of β-actin levels to normalize possible differences between each loading volume. The protein levels were presented as percentage changes compared with control group, designated as 100%. One-way ANOVA was performed followed by a post-hoc Tukey's Multiple Comparison Test where applicable for inter-group comparison, with $P<0.05$ considered a significant difference. The result showed that acute Δ^9THC treatment at all doses modulates the levels of ERK1, ERK2 ($P<0.01$),

p-ERK1 ($P<0.05$; $P<0.01$), p-ERK2 ($P<0.01$), CREB and *c-fos* ($P<0.05$; $P<0.01$) proteins. However, only Δ^9THC at 0.5 mg/kg modulates the level of p-CREB. These finding produce inconclusive result and future investigation needs to be explored. Meanwhile, for chronic treatment, administration of Δ^9THC also modulates the levels of ERK1 ($P<0.05$; $P<0.001$), ERK2 ($P<0.01$), CREB, p-CREB ($P<0.01$) and *c-fos* ($P<0.05$) protein levels. There have a reduction in the level of p-ERK1 at Δ^9THC 1.0 mg/kg. Generally, it supposed to be increased since it had been noticed that Δ^9THC at 2.0 mg/kg showed significantly differences. The mechanism underlying these finding is remain unclear and considered as inconclusive result. The protein expression was also studied to determine whether the changes observed due to CB_1 receptor activation using selective antagonist SR141716A. Based on the result obtained, pre-treatment with CB_1 antagonist SR141716A failed to alter the Δ^9THC induced effect on ERK1 and *c-fos* expressions. Interestingly, these effects can be reversed by SR141716A on ERK2, p-ERK1, p-ERK2 and p-CREB proteins. In conclusion, the present data suggest that synthesis of ERK1 and *c-fos* are not CB_1 mediated effect but synthesis of ERK2 and activated of ERK as well as CREB by Δ^9THC are through CB_1 mediated effect pathway.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN PERANTARA CB_1 DELTA-9-TETRAHYDROCANNABINOL (Δ^9THC) TERHADAP PARAS PROTEIN DI DALAM HIPOKAMPUS TIKUS *SPRAGUE DAWLEY* JANTAN

Oleh

FATIN NADZIRAH BINTI ZAKARIA

Januari 2013

Pengerusi : Mohamad Aris Bin Mohd Moklas, PhD

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Neuroplastisiti adalah merujuk kepada keupayaan otak untuk bertindak balas sebagai hasil daripada pengalaman yang tertentu. Delta-9-tetrahydrocannabinol (Δ^9THC), diekstrak secara semulajadi daripada tumbuhan *Cannabis sativa* spesis betina yang dianggap sebagai bahan psikotropik yang paling aktif. *Cannabis sativa* merupakan tumbuhan yang tumbuh dalam iklim tropika dan zon iklim sederhana. Δ^9THC telah terbukti mempengaruhi deria persepsi, kognitif dan ingatan, ganjaran, selera, koordinasi motor dan penumpuan. Rujukan kajian terkini menunjukkan bahawa Δ^9THC memainkan peranan yang penting dalam keplastikan sinaps. Kajian *in-vivo* dan *in-vitro* menunjukkan bahawa suntikan Δ^9THC memodulasi beberapa protein dimana ia terlibat dalam keplastikan sinaps di bahagian hipokampus. Kajian terdahulu memberi petunjuk tentang bagaimana hipokampus memainkan peranan yang penting di dalam proses ingatan. Walaubagaimanapun, pengubahsuaian molekul dan mekanisma sel yang

membawa kepada keplastikan sinaps masih belum difahami sepenuhnya. Justeru, objektif kajian ini adalah untuk menilai kesan rawatan Δ^9THC akut dan kronik bagi ERK1 dan ERK2 (p44 MAP kinase), p-ERK1 dan p-ERK2 (p-p44 dan p-p42 MAP kinase), CREB, p-CREB dan *c-fos* dalam hipokampus tikus dengan mengkaji mekanisme reseptor CB_1 termasuk reseptor agonis dan reseptor antagonis. Protein-protein ini dianggap terlibat dalam keplastikan sinaps. Kajian ini dilakukan dalam 3 eksperimen. Bagi eksperimen 1 dan 2, tikus telah dibahagikan kepada empat kumpulan iaitu kumpulan kawalan dan 3 kumpulan yang dikaji (0.5, 1.0 and 2.0 mg/kg). Untuk rawatan akut (eksperimen 1), tikus dalam kumpulan kawalan menerima ejen pelarut (2% ethanol + 0.9% NaCl) sahaja manakala bagi kumpulan kajian, tikus menerima 0.5, 1.0 dan 2.0 mg/kg Δ^9THC dan telah disuntik setiap 48 jam selama 7 hari. Untuk rawatan kronik (eksperimen 2), Δ^9THC (0.5, 1.0 dan 2.0 mg/kg) telah disuntik setiap 48 jam selama 21 hari. Semua bahan aktif dan ejen pelarut telah diberi melalui suntikan intra peritoneum (i.p). Jumlah suntikan i.p adalah 0.1 ml/100 g daripada berat badan. Berdasarkan kajian kronik, 2.0 mg/kg Δ^9THC yang merupakan dos optimum telah digunakan dalam kajian gabungan (eksperimen 3) untuk mengkaji kesan kronik Δ^9THC dengan pra-rawatan CB_1 antagonis SR141716A (rimonabant). Semua larutan bahan aktif disediakan sejurus sebelum eksperimen. Data diperbetulkan berdasarkan paras β-aktin untuk menormalkan perbezaan yang mungkin diantara setiap isipadu muatan. Paras protein telah diterjemahkan sebagai perubahan peratusan berbanding dengan kumpulan kawalan, yang ditetapkan sebagai 100%. One-way ANOVA telah dijalankan diikuti dengan Ujian Perbandingan Pelbagai Tukey pos-hoc dimana ia digunapakai untuk perbandingan antara kumpulan, dengan $P<0.05$ dianggap sebagai perbezaan yang signifikan. Hasil menunjukkan bahawa rawatan akut Δ^9THC pada setiap dos

memodulasi paras protein ERK1, ERK2 ($P<0.01$), p-ERK1 ($P<0.05$, $P<0.01$), p-ERK2 ($P<0.01$), CREB dan *c-fos* ($P<0.05$, $P<0.01$). Walaubagaimanapun, hanya Δ^9THC dos 0.5 mg/kg memodulasi paras protein p-CREB. Penemuan ini menghasilkan keputusan yang tidak dapat disimpulkan dan kajian lanjutan perlu dijalankan. Sementara itu, untuk rawatan kronik, suntikan Δ^9THC juga memodulasi paras protein ERK1 ($P<0.05$, $P<0.001$), ERK2 ($P<0.01$), CREB, p-CREB ($P<0.01$) and *c-fos* ($P<0.05$). Terdapat penurusan paras protein p-ERK1 pada dos 1.0 mg/kg Δ^9THC. Secara umum, paras protein tersebut sepatutnya meningkat apabila terdapat perbezaan yang signifikan pada dos 2.0 mg/kg Δ^9THC. Mekanisma di sebalik penemuan ini masih tidak jelas dan dianggap sebagai keputusan yang tidak dapat disimpulkan. Ekspresi protein juga dikaji untuk menentukan samada perubahan yang diperhatikan adalah melalui pengaktifan reseptor CB_1 menggunakan antagonis SR141716A. Berdasarkan keputusan yang diperolehi, pra-rawatan dengan CB_1 antagonis SR141716A gagal mengubah kesan Δ^9THC yang teraruh pada protein ERK1 dan *c-fos*. Menariknya, kesan ini boleh diterbalikkan oleh SR141716A pada protein ERK2, p-ERK1, p-ERK2 dan p-CREB. Kesimpulannya, sintesis ERK1 dan *c-fos* adalah bukan kesan perantara CB_1, akan tetapi, sintesis ERK2, pengaktifan ERK dan juga CREB oleh Δ^9THC adalah melalui kesan perantara CB_1.

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APPROVAL

I certify that an Examination Committee has met on 09/01/2013 to conduct the final examination of Fatin Nadzirah Binti Zakaria on her Master of Science thesis entitled "CB_1-mediated effect of Delta-9-tetrahydrocannabinol (Δ^9THC) on neuronal protein expressions in the hippocampus of male *Sprague dawley* rats" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian (Higher Degree) Act 1981. The committee recommends that the student be awarded the degree of Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for the quotations and citations which have been duly acknowledge. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or at any other institution.

FATIN NADZIRAH BINTI ZAKARIA

Date: 9 January 2013

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