Endocannabinoid System: behavioral modulation in murine models by Cannabinoids type 2 receptors

Sistema endocanabinóide: modulação comportamental em modelos murinos por receptores de canabinóides tipo 2

Sistema endocannabinoide: modulación del comportamiento en modelos murinos por receptores cannabinoides tipo 2

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Abstract
In the last decades, the eCB system has been highlighted by its neuro and immunomodulatory effects. Beyond CB_1R effects in Central Nervous System (CNS), CB_2R target drugs has been showed to be promising to mitigation of neuroinflammatory diseases in mouse models. However, it remains unknow the effects of CB_2R target drugs on behavior. Therefore, we review the effects of CB_2R on behavior in murine models by Pubmed website, selecting studies between 2001 to 2021. In this sense, many studies has demonstrated the effects of overexpression, lack, activation or antagonization of CB_2R on Aggressive behavior, Memory-associated behaviors, Mood disorders and Reward behavior. Similarly, it is not clear yet how the eCB system modulates the behavior through CB_2Rs present in neurons. Thus, in mouse models, although the pharmacological treatment with CB_2R target drugs seems to be promising for neuroinflammatory diseases, on behavior there are few answers about the pathways of this modulation, as well as, it is fundamental the development and/or the update of behavioral tests that evaluate many parameters, then expose better interpretations in these tests.
Keywords: Cannabinoid receptor type 2; Endocannabinoid system; Behavioral modulation; Mood disorders; CB_2R target drugs; Behavioral tests.

Resumo
Nas últimas décadas, o sistema eCB tem se destacado por seus efeitos neuro e imunomoduladores. Além dos efeitos do RCB_1 no Sistema Nervoso Central (SNC), os medicamentos alvo do RCB_2 têm se mostrado promissores na mitigação de doenças neuroinflamatórias em modelos de camundongos. No entanto, permanecem desconhecidos os efeitos das drogas alvo do RCB_2 no comportamento. Portanto, revisamos os efeitos do RCB_2 no comportamento em modelos murinos pelo site Pubmed, selecionando estudos entre 2001 a 2021. Nesse sentido, muitos estudos têm demonstrado os efeitos da superexpressão, falta, ativação ou antagonização do RCB_2 no Comportamento agressivo, nos Comportamentos associados à memória, nos Transtornos de humor e no Comportamento de recompensa. Da

mesma forma, ainda não está claro como o sistema eCB modula o comportamento por meio dos RCB$_2$s presentes nos neurônios. Assim, em modelos de camundongos, embora o tratamento farmacológico com fármacos alvo do RCB$_2$ pareça promissor para doenças neuroinflamatórias, sobre o comportamento existem poucas respostas sobre as vias desta modulação, bem como, é fundamental o desenvolvimento e/ou atualização de testes comportamentais que avaliam muitos parâmetros e, por conseguinte, expõem melhores interpretações nesses testes.

Palavras-chave: Receptor canabinóide do tipo 2; Sistema endocanabinóide; Modulação comportamental; Transtornos de Humor; Drogas alvo de RCB$_2$; Testes comportamentais.

Resumen

En las últimas décadas, el sistema eCB se ha destacado por sus efectos neuro e inmunomoduladores. Más allá de los efectos de RCB$_1$ en el Sistema Nervioso Central (SNC), se ha demostrado que los fármacos diana de RCB$_2$ son prometedores para la mitigación de enfermedades neuroinflamatorias en modelos de ratón. Sin embargo, se desconocen los efectos de los fármacos diana RCB$_2$ sobre el comportamiento. Por ello, revisamos los efectos del RCB$_2$ sobre el comportamiento en modelos murinos por la web Pubmed, seleccionando estudios entre 2001 y 2021. En este sentido, numerosos estudios han demostrado los efectos de la sobreexpresión, falta, activación o antagonización del RCB$_2$ sobre el comportamiento agresivo, Memory- comportamientos asociados, trastornos del estado de ánimo y comportamiento de recompensa. Del mismo modo, aún no está claro cómo el sistema eCB modula el comportamiento a través de los RCB$_2$ presentes en las neuronas. Así, en modelos de ratón, aunque el tratamiento farmacológico con fármacos diana RCB$_2$ parece prometedor para las enfermedades neuroinflamatorias, sobre el comportamiento existen pocas respuestas sobre las vías de esta modulación, así como, es fundamental el desarrollo y/o la actualización de pruebas de comportamiento que evalúan muchos parámetros, luego exponen mejores interpretaciones en estas pruebas.

Palabras clave: Receptor de cannabinoides tipo 2; Sistema endocannabinoide; Modulación del comportamiento; Trastornos del estado de ánimo; Fármacos diana RCB$_2$; Pruebas de comportamiento.

1. Introduction

Endocannabinoid System

The Endocannabinoid (eCB) system raised in the academic world in the end of the last century, since then its modulatory effects on other systems have been highlighted, mainly by its neuro and immunomodulatory effects.

The eCB system is characterized mainly by two cannabinoid receptors: type 1 (CB$_1$R) and type 2 (CB$_2$R). Both are G protein-coupled, which CB$_1$R was mainly linked to neural modulation, whereas CB$_2$R was mainly linked to immune modulation (Castillo, Younts, Chavez, & Hashimotodani, 2012; Piomelli, 2003). However, more recently it is clear a double receptor modulation in immune and nervous system by both CB$_1$ and CB$_2$ receptors (Borner, Smida, Hollt, Schraven, & Kraus, 2009; McCoy, 2016; Naguib et al., 2012; Stella, 2010). Here, our review focuses mainly CB$_2$R effects on behavioral modulation in mouse models.

In this sense, the immunomodulatory effect of activation of CB$_2$R by the activation of Protein Kinase A in immunological, epithelial (Basu & Dittel, 2011; Borner et al., 2009; Persidsky et al., 2015), and glial cells (Ehrhart et al., 2005; Merighi, Gessi, Varani, Fazzi, et al., 2012; Merighi, Gessi, Varani, Simioni, et al., 2012) has been promising to many inflammatory diseases, such as obesity (Q. Wu et al., 2020), Parkinson disease (Concannon, Okine, Finn, & Dowd, 2015; Price et al., 2009), Sepse (Tschop et al., 2009), Stroke (Ronca et al., 2015), and Alzheimer disease (J. Wu et al., 2013). In addition, the activation of CB$_2$R as a pharmacological target to neuroinflammatory diseases is an alternative against the psychotropic effects of the activation of CB$_1$R within the attributions of eCB system: the side effects of *marihuana* (*Cannabis*) use are mainly due to CB$_1$R activation by THC (Δ9-tetrahydrocannabinol) (Hashiesh et al., 2020; Volkow, Baler, Compton, & Weiss, 2014).

In contrast, the presence of CB$_2$R in neurons has motivated the research for neuromodulators effects of the systemic activation of CB$_2$R to know the possibility of side effects, as well as to know the possibility of treatment of mood disorders, such as anxiety and depression (Chen, Gao, Gao, Su, & Wu, 2017). Therefore, the neuromodulators effects of eCB System by CB$_2$R remain unknow, mainly in behavior. Thus, our goal was review and systematize the CB$_2$R effects on behavioral

modulation in murine models.

2. Methodology

The source of this review comprehends studies from PubMed website, which the keyworks was: "Cannabinoid receptor type 2"; "Endocannabinoid system"; and "Behavioral test". Thus, the first step was the select of studies between 2001 to 2021. Our goal was select experimental studies that have used behavioral tests in mouse models with overexpression, lack, activation or antagonization of CB_2R, which includes CB_2R target drugs (agonists and/or antagonists), CB_2R-knockout mice, overexpression or lack of CB_2R by other methodologies. The second step was select the main literature studied in behavioral clusters. The last step was including additional studies to explain our view point about our conclusions overall. According to Estrela, (2018) our study can be considerable a Narrative Revision (Estrela, 2018).

3. Results and Discussion

During our search, we systematize 12 methodologies, 33 effects/outcomes from 10 studies in 4 behavioral clusters: Aggressive behavior, Memory-associated behavior, Mood disorders and Reward behavior. Furthermore, some effects/outcomes appear more than once and can be linked to more than one of the 4 behavioral clusters (Table 1).

Aggressive behavior

Aggressiveness could be found in any mammal, likewise it stands as an important preserved behavior since mouse to humans. In this sense, Rodriguez-Arias, et al (2013) demonstrated that CB_1R could be present in aggressive modulation in mice model (Rodriguez-Arias et al., 2013). Similarly, later the same group also showed that CB_2R-knockout mice are more aggressive than wild type (WT), whereas acute administration of CB_2R-agonist attenuate the aggressiveness in WT after isolation, while the CB_2R-antagonist reversed it, in addition the CB_2R-knockout mice did not have its aggressiveness changed by isolation, indicating a modulatory effect on aggressive behavior by CB_2R (Rodriguez-Arias et al., 2015). Thus, supporting the eCB system as an aggressive behavior modulator by both CB_1 and CB_2 receptors.

Memory-associated behavior

Memory could determinates our decisions and consequently our behavior. Therefore, murine models have been used to quantify it by behavioral tests. In this sense, Garcia-Gutierrez, et al (2013) reported an impairment in the short and long term contextual memory in CB_2R-knockout mice and in CB_2R-antagonist acute treatment, while the CB_2R-agonist acute treatment improved it in Step-down inhibitory avoidance test (SDIA), however it was not clear if these differences are in contextual memory itself or in the exploratory behavior: CB_2R-knockout mice, for example, expressed difference against WT in the train part of SDIA in this study, suggesting then an exploratory behavior modulation which may have confounded the results (Garcia-Gutierrez et al., 2013).

Similarly, Lim and Kim (2016a) had controversial results to linked the lack of CB_2R to contextual memory either, CB_2R-knockout mice showed a contextual memory impairment by decreased of freezing in contextual test without difference in cued test of Fear conditioning behavior test (FCBT), as well as the CB_2R antagonist acute administration did not promote any difference in FCBT or Y-maze test (YMT), furthermore CB_2R-knockout mice showed increased in spontaneous alteration in YMT (Li & Kim, 2016a). Later, the same group brought out an elegant study, they reported that the overexpression of CB_2R in pyramidal cells or interneurons from CA1 area of hippocampus did not promote any change in YMT, FCBT or Novel object test (NOT), whereas the lack of CB_2R in pyramidal cells from the same area by CRISPR (Clustered regularly interspaced short

palindromic repeats) promotes increased in spontaneous alteration in YMT, as well as, by the same methodologies, the overexpression of CB_2R in microglial cells from CA1 area of hippocampus promotes increased of freezing in contextual text of FCBT, whereas the lack of CB_2R promotes a decreased of freezing in the same test, however this lack also promotes an improvement in NOT (Li & Kim, 2017). Indicating then, an improving of spatial work memory by the lack of CB_2R, however it is not clear yet a CB_2R modulation on fear memory or spatial memory, as well as it also not clear if that modulation is mediated by neurons or microglial cells.

In addition, in mice models of neural diseases, the CB_2R-agonist chronic treatment has not showed any difference in spatial memory in Morris water maze test (MWM) in Alzheimer's disease model (J. Wu et al., 2013), and has showed a memory-associated recognition impairment in NOT in a stroke model (Ronca et al., 2015). Thus, despite the CB_2R-agonist treatment attenuate damages in these and others neural diseases in mice models, it has not showed good outcomes in memory-associated behavior. In the other hand, the eCB system stands present in memory-associated behavior, especially in fear-associated behavior and this modulation is more associated with the CB_2Rs present in microglial cells, thus it must to be considerate in adoption of treatments with CB_2R target drugs.

Mood disorders

Beyond the neuroinflammatory diseases, the eCB system has been also investigated over the mood disorders, which the murine models are large used in these models. Thereby, García-Gutiérrez, et al (2012) has showed that synthetic CB_2R-antagonist acute-treatment has an anxiogenic effect, whereas the synthetic CB_2R-agonist acute-treatment has no effect in Light-Dark box (LDB), but reversed the effect of CB_2R-antagonist acute-treatment, interestingly the chronic treatments has reversed these effects: the agonist induced an anxiogenic effect and the antagonist induced an anxiolytic effect, which these last effects linked to an expression changes of GABAergic receptors on the cortex and on the Amidala (Garcia-Gutierrez, Garcia-Bueno, Zoppi, Leza, & Manzanares, 2012). Similarly, the increased time in central area of Open field test (OFT) or open arm of Elevated plus-maze (EPM) promoted after β-Caryophyllene (BCP) acute-treatment, a CB_2R-agonist, was reported as an anxiolytic effect dependent of CB_2Rs in these tests (Bahi et al., 2014). Indeed, this increased or decreased in the center of OFT, or in the open arm of EPM, or in the light area of LDB, has reported as an anxiolytic or anxiogenic effect respectively.

In the other hand, Li & Kim (2016a) has showed that CB_2R-knockout mice and CB_2R-agonist acute-treatment does not have effect in the exploratory behavior into aversive areas of typical tests of anxiety-like behavior (Li & Kim, 2016a). However, the use of knockout mice to research for eCB system roles is questionable because eCB system is present in Central nervous system (CNS) development (Harkany et al., 2007). In addition, it is unclear whether the typical tests of anxiety-like behavior in mouse models, such as OFT, EPM, LDB and Zero-maze test (ZMT) evaluate precisely this behavior (Ennaceur & Chazot, 2016). Moreover, recently it has showed that the overexpression of CB_2Rs in glutamatergic neurons from CA1 area of hippocampus also induced increased of activity in the central area of OFT, without alteration in ZMT (Li & Kim, 2017). It suggests that the increased of exploratory activity in aversive area of OFT could be does not associated with anxiety-like behavior. Thus, it must to be more investigated.

Furthermore, over the depression-like behavior, Bahi, et al (2014) also demonstrated that BCP acute-treatment decreased the latency to eat in Novelty suppressed feeding test (NSFT), and decreased the immobility time in Forced swim test (FST) and in Tail suspension test (TST) in mice model, whereas the action of acute-treatment with CB_2R-antagonist reversed these results (Bahi et al., 2014). It supports the use of CB_2R as a pharmacological target to mood disorders, however there are few data to corroborate it yet. Nonetheless, the BCP treatment has other targets beyond CB_2R (Gertsch et al., 2008; Hashiesh et al., 2020), which means it is necessary an investigation for its outcomes in mice models of depression-like behavior.

Table 1. The effects/outcomes of CB_2R on behavioral tests in mouse models.

Aggressive behavior		
Methodology	**Effects/Outcomes**	**Reference**
Systemic CB₂R-agonist acute-treatment	Decreased of the aggressiveness after isolation	Rodriguez-Arias et al., 2015
Systemic CB₂R-antagonist acute-treatment	Reversed the effect of agonist	
CB₂R-knockout mice	Increased of aggressiveness	
	It did not promote any difference in aggressiveness after isolation	

Memory-associated behavior		
Methodology	**Effects/Outcomes**	**Reference**
Systemic CB₂R-agonist acute-treatment	Improvement in the short and long term contextual memory	Garcia-Gutierrez et al., 2013
Systemic CB₂R-antagonist acute-treatment	Impairment in the short and long term contextual memory	
CB₂R-knockout mice		
Systemic CB₂R-antagonist acute-treatment	It did not promote any difference in FCBT or YMT	Li & Kim, 2016a
CB₂R-knockout mice	Impairment in contextual memory by decreased of freezing in contextual test without difference in cued test of FCBT	
	Increased in spontaneous alteration in YMT	
Overexpression of CB₂R in pyramidal cells from CA1 area of hippocampus	It did not promote any change in YMT, FCBT or NOT	Li & Kim, 2017
Overexpression of CB₂R in interneurons from CA1 area of hippocampus		
Lack of CB₂R in pyramidal cells from CA1 area of hippocampus by CRISPR	Increased in spontaneous alteration in YMT	
Overexpression of CB₂R in microglial cells from CA1 area of hippocampus	Increased of freezing in contextual text of FCBT	
Lack of CB₂R in the in microglial cells from CA1 area of hippocampus by CRISPR	Decreased of freezing in contextual text of FCBT	
	Improvement in memory-associated recognition in NOT	
Systemic CB₂R-agonist chronic-treatment	It did not promote effects in MWM	J. Wu et al., 2013
	Impairment in memory-associated recognition in NOT	Ronca et al., 2015

Mood disorders		
Methodology	**Effects/Outcomes**	**Reference**
Systemic CB₂R-antagonist acute-treatment	Decreased the time in light box of LDB	Garcia-Gutierrez, Garcia-Bueno, Zoppi, Leza, & Manzanares, 2012
Systemic CB₂R-agonist acute-treatment	Reversed the effect of antagonist	
CB₂R-antagonist chronic-treatment	Increased the time in aversive areas of LDB and EPM	
Systemic CB₂R-agonist chronic-treatment	Decreased the time in aversive areas of LDB and EPM	
CB₂R-knockout mice	It did not promote any effect in OFT or ZMT	Li & Kim, 2016a
Systemic CB₂R-agonist acute-treatment		
Systemic CB₂R-antagonist acute-treatment	Reversed the effects of agonist	
Overexpression of CB₂R in glutamatergic neurons from CA1 area of hippocampus	Increased of activity in the central area of OFT	Li & Kim, 2017
	It did not promote any effect in ZMT	
Systemic CB₂R-agonist acute-treatment	Increased the time in aversive areas of OFT and EPM	Bahi et al., 2014
	Decreased the latency to eat in NSFT	
	Decreased the immobility time in FST and in TST	

Reward behavior		
Methodology	**Effects/Outcomes**	**Reference**
Systemic, intranasal or intra-accumbens CB₂R-agonist acute-treatment	Inhibits the cocaine self-administration	Xi et al., 2011
	Inhibits the hyper activity induced by cocaine	
Systemic CB₂R-agonist acute-treatment	Increased of activity in the central area of OFT	Bahi et al., 2014
Overexpression of CB₂R in glutamatergic neurons from CA1 area of hippocampus		Li & Kim, 2017
Systemic CB₂R-agonist acute-treatment	Inhibits the hyper activity induced by cocaine	Lopes, Bastos, Costa, Aguiar, & Moreira, 2020
Systemic CB₂R-agonist chronic-treatment	Reversed the effect of agonist	

Source: Authors (2021).

Reward behavior

The eCB system has been showed in mesolimbic pathway, thus in the modulation of reward behavior, which is a fundamental target in the mitigation of side effects from psychoactive drugs. In this sense, the chronic activation of CB_2Rs in

the hippocampus increased synaptic transmission (Kim & Li, 2015). Completely, CB_2R-kockout mice has decreased of excitatory synapse in the hippocampus (Li & Kim, 2016b). As well as, the strength of hippocampus nucleus-accumbens (NAc) synapse drives conditioned place preference, similarly as the use of anti-depressive drugs is linked to the increased of this pathway, and differently as the chronic stress is linked to the decreased of this pathway (LeGates et al., 2018). The NAc has an important role in mesolimbic pathway about the regulation of dopamine levels, motor activity and NAc is also linked to behavior changes induced by drugs abuse intake, however NAc roles remains unknown, but the hippocampus-NAc input has showed important modulatory effect in reward behavior (Perez & Lodge, 2018; Scudder, Baimel, Macdonald, & Carter, 2018).

Therefore, we suggest that the increased in central area of OFT by CB_2R-acute treatment (Bahi et al., 2014) or overexpression of CB_2Rs in glutamatergic neurons from CA1 area of hippocampus (Li & Kim, 2017) would be linked to a eCB system neuro-modulatory effect in reward behavior by an increase of exploration, instead of anxiety-like behavior.

In addition, the eCB system has also linked to mesolimbic pathway by presence of CB_2Rs in dopaminergic neurons of ventral tegmental area (Zhang et al., 2014). Completely, the systemic, the intranasal or the intra-accumbens acute treatment with CB_2R-agonist inhibits the cocaine self-administration and inhibits the increased of hyper activity induced by cocaine, whereas this drug effect is absent in CB_2R-knockout mice (Xi et al., 2011). Similarly, Lopes, et al (2019) also demonstrated that acute administration of CB_2R-agonist inhibits hyper activity induced by cocaine, whereas the CB_2R-antagonist reversed it (Lopes, Bastos, Costa, Aguiar, & Moreira, 2020). It supports an interesting application in mitigation of side effects of abuse drugs by pharmacological treatment of CB_2R target drugs.

4. Conclusion

In summary, after few years of eCB system discovered, there are a range of studies and reviews about its modulatory effect on CNS, and it is clear a large potential of the use of eCB system as a pharmacological target for many diseases, especially in immune and neural diseases, however, it remains unknow these effects on behavior. In this review we systematize 12 methodologies, 33 effects/outcomes from 10 studies in 4 behavioral clusters (Table 1). Thus, we conclude the eCB system modulates behaviors by CB_2R in mouse models, which includes Aggressive behavior, Memory-associated behavior, Mood disorders and Reward behavior, as well as, the CB_2R target drugs has a large potential to be a pharmacological treatment in Mood disorders and mitigation of side effects promoted by abuse drugs. However, in spite of a considerable number of studies that linked the presence, the lack, the activation or the antagonization of CB_2R to behavioral changes, there are a few answers about which other behaviors could be modulated by it, as well as, there is not sure about all consequences of acute or chronic treatment with agonists or antagonists of CB_2R. This lack of answers is promoted both by the complexity of eCB system and the controversial interpretations in behavioral tests. In this sense, it is necessary future researches to multi target treatments into eCB system, including receptor side effects and physiology and pharmacology behavioral effects modulated by CB_2R. As well as, the development and/or the update of behavioral tests that can evaluate the correlation of behavioral parameters and eCB system should be provide.

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