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Cannabinoid-induced motor dysfunction *via* autophagy inhibition

Cristina Blázquez^{1,2}, Andrea Ruiz-Calvo^{1,2}, Raquel Bajo-Grañeras^{1,2},
Jerome M. Baufreton³, Eva Resel^{1,2}, Marjorie Varilh⁴, Antonio C. Pagano Zottola⁴,
Yamuna Mariani⁴, Astrid Cannich⁴, José A. Rodríguez-Navarro², Giovanni Marsicano⁴,
Ismael Galve-Roperh^{1,2}, Luigi Bellocchio^{4,*}, Manuel Guzmán^{1,2,*}

¹Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Instituto Universitario de Investigación Neuroquímica (IUIN) and Department of Biochemistry and Molecular Biology, Complutense University, 28040 Madrid, Spain

²Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), 28034 Madrid, Spain

³Centre National de la Recherche Scientifique (CNRS) and University of Bordeaux, Institut des Maladies Neurodégénératives, UMR5293, 33076 Bordeaux, France

⁴Institut National de la Santé et de la Recherche Médicale (INSERM) and University of Bordeaux, NeuroCentre Magendie, Physiopathologie de la Plasticité Neuronale, U1215, 33077 Bordeaux, France

*Corresponding authors:

Manuel Guzmán. Department of Biochemistry and Molecular Biology, School of Biology, Complutense University, 28040 Madrid, Spain. Telephone: +34913944668. Email: mguzman@quim.ucm.es

Luigi Bellocchio. INSERM, NeuroCentre Magendie, Physiopathologie de la Plasticité
Neuronale, U1215, 33077 Bordeaux, France. Phone: +33557573754; Email:
luigi.bellocchio@inserm.fr

ACCEPTED MANUSCRIPT

ABSTRACT

The recreational and medical use of cannabis is largely increasing worldwide. Cannabis use, however, can cause adverse side effects, so conducting innovative studies aimed to understand and potentially reduce cannabis-evoked harms is important. Previous research conducted on cultured neural cells had supported that CNR1/CB₁R (cannabinoid receptor 1), the main molecular target of cannabis, affects macroautophagy/autophagy. However, it was not known whether CNR1 controls autophagy in the brain *in vivo*, and, eventually, what the functional consequences of a potential CNR1-autophagy connection could be. We have now found that Δ^9 -tetrahydrocannabinol (THC), the major intoxicating constituent of cannabis, impairs autophagy in the mouse striatum. Administration of autophagy activators (specifically, the rapalog temsirolimus and the disaccharide trehalose) rescues THC-induced autophagy inhibition and motor dyscoordination. The combination of various genetic strategies *in vivo* supports the idea that CNR1 molecules located on neurons belonging to the direct (striatonigral) pathway are required for the autophagy- and motor-impairing activity of THC. By identifying autophagy as a mechanistic link between THC and motor performance, our findings may open a new conceptual view on how cannabis acts in the brain.

KEY WORDS

Autophagy; cannabinoid; drug abuse; motor behavior; MTOR; striatum

Cannabis is the third most popular drug of abuse in the world following alcohol and tobacco, and its use is rapidly increasing. To date, four countries in the world and a dozen states in the USA have legalized the recreational use of cannabis. In addition, medicinal-cannabis dispensation programs have been implemented in approximately thirty countries globally, as well as in a similar number of states in the USA, and several cannabinoid-based medicines have been approved by foremost regulatory agencies (e.g., FDA, EMA and Health Canada) as anti-emetic, anti-cachexic, analgesic and anti-spastic adjuvants. However, cannabis use is associated with several undesired and possibly dangerous side effects, so it is crucial that innovative procedures aimed to understand and potentially reduce cannabis-evoked harms are explored. Δ^9 -Tetrahydrocannabinol (THC), the key intoxicating constituent of cannabis, exerts its biological effects mainly by activating CNR1/CB₁R (cannabinoid receptor 1), one of the most abundant metabotropic receptors in the mammalian central nervous system. This receptor is particularly expressed in discrete brain areas involved in the control of learning and memory (cortex, hippocampus), motor behavior (striatum, cerebellum), emotions (amygdala) and autonomic and endocrine functions (hypothalamus, pons, medulla), therefore modulating a wide plethora of biological processes. Recent evidence had suggested that CNR1 controls autophagy in cultured neural cells. Strikingly, however, in some cellular settings cannabinoids *via* CNR1 enhance autophagy, whereas in others they inhibit autophagy. Moreover, it was not known whether CNR1 controls autophagy in the brain *in vivo*, and, eventually, what the functional consequences of a potential CNR1-autophagy connection could be.

In a recent study [1] we have explored the effect of THC on autophagy in the mouse brain. A single injection of the drug (at 10 mg/kg body weight) concertedly increases LC3-II and SQSTM1/p62 levels in the striatum but not in other representative

brain regions such as the cortex, the hippocampus and the cerebellum. THC also increases LC3-II and SQSTM1 levels in primary cultures of mouse striatal neurons. In contrast, when an accumulation of LC3-II and SQSTM1 is achieved by incubating the cells with lysosomal inhibitors, THC is unable to heighten those autophagy protein levels further. Hence, THC seems to inhibit autophagy in striatal neurons both *in vivo* and *in vitro*.

Next, we studied the biological impact of this THC-evoked inhibition of striatal autophagy. For this purpose, mice were subjected to tests of motor behavior, an archetypical process that is controlled by the striatum and affected by cannabis. As expected, THC impairs motor coordination, as determined by the RotaRod test, and motor activity, as determined by the open-field test. Strikingly, boosting striatal autophagy either by pharmacological manipulation (with the rapalog temsirolimus) or dietary intervention (with the disaccharide trehalose) rescues THC-induced motor dyscoordination but not THC-induced motor inactivity, thus indicating that autophagy selectively modulates the coordination component of overall motor behavior.

What may be the neuroanatomical substrate of the observed THC effects? First, we proved the participation of CNR1 by systemically administering the CNR1-selective antagonist SR141716 (rimonabant) to wild-type mice. Second, we found that the THC-evoked inhibition of striatal autophagy and motor coordination is not evident in mice in which the gene encoding CNR1 had been knocked out in striatal neurons belonging to the direct (striatonigral) pathway. Third, in contrast, we observed that mice in which the gene encoding CNR1 had been knocked out in corticostriatal projections (which are usually considered a key determinant of striatal activity) are fully responsive to the THC-evoked inhibition of striatal autophagy and motor coordination. And fourth, an array of experiments conducted on mice selectively expressing (A) reporter genes for direct-

pathway and indirect-pathway (striatopallidal) neurons, (B) dominant-negative RPTOR/raptor in direct-pathway neurons, or (C) SQSTM1 in direct-pathway neurons, provide further support to the selective participation of CNR1 molecules located on neurons belonging to the striatal direct pathway in the autophagy-inhibiting and motor-dyscoordinating activity of THC.

Taken together, these findings indicate that impairment of autophagy may be a new mechanism involved in at least some cannabinoid-induced motor alterations. On molecular grounds, our data would favor a “two-hit” model by which engagement of CNR1 may impair autophagy. First, CNR1 activation, through its well-known coupling to the phosphoinositide 3-kinase-AKT-MTORC1 pathway, could lead to ULK1 phosphorylation, which, subsequently, would inhibit autophagosome formation/autophagy initiation. Second, CNR1 activation, by a hitherto undefined mechanism that may conceivably involve an impact on lysosomal function, would inhibit autophagosome clearance/autophagy completion (Figure 1).

We are aware, however, that our work has several shortcomings that could limit the generalization of its conclusions to other experimental conditions (*e.g.*, other types and doses of cannabinoids, times of cannabinoid treatment, and behavioral traits). We also note that our work does not unveil the precise cellular and molecular mechanisms by which the CNR1-evoked inhibition of autophagy in striatal direct-pathway neurons affects brain functionality to affect motor coordination. Nonetheless, neuronal communication is sensitive to the status of both the MTORC1 pathway and proteostatic processes such as autophagy, which, for example, may clear dysfunctional proteins and fine-tune the trafficking/recycling of membrane neurotransmitter receptors. Hence, it is conceivable that the control of neurotransmission exerted by CNR1 on the striatal direct pathway might be mechanistically connected to the THC-evoked effects on MTORC1

and autophagy shown in our study. Further research will be necessary to deepen into the mechanistic and biological details of cannabinoid anti-autophagic action. These issues notwithstanding, our findings might be applicable not only to motor behavior but also to other neurobiological processes that are controlled by the striatum and affected by cannabis (e.g., cognition, affection and reward). Moreover, from a translational standpoint, our data add to previous reports suggesting that targeting the MTORC1 pathway (and now, as shown in our study, autophagy) might provide a rationale for designing strategies aimed to manage some particular behavioral alterations induced by cannabis consumption.

DISCLOSURE STATEMENT

We declare no competing financial interests.

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REFERENCE

[1] Blázquez C, Ruiz-Calvo A, Bajo-Grañeras R, Baufreton JM, Resel E, Varilh M, Pagano Zottola AC, Mariani Y, Cannich A, Rodríguez-Navarro JA, Marsicano G, Galve-Roperh I, Bellocchio L, Guzmán M. Inhibition of striatonigral autophagy as a link between cannabinoid intoxication and impairment of motor coordination. *Elife*. 2020;9:e56811.

FIGURE LEGEND

Figure 1. Scheme depicting the impact of THC on striatal autophagy and motor coordination. THC, the main intoxicating component of cannabis, would bind to CNR1 molecules located on neurons belonging to the striatal direct (striatonigral) pathway. Upon engagement, CNR1, by (i) activating the MTORC1 pathway and (ii) blocking autolysosomal function, would inhibit autophagy. This, in turn, would impair motor coordination by a hitherto unknown mechanism.

