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Research Report

CB1 receptor knockout mice show similar behavioral modifications to wild-type mice when enkephalin catabolism is inhibited

Fanny Jardinaud[a], Dominique Crété[a], Corinne Canestrelli[a], Catherine Ledent[b], Bernard P. Roques[a], Florence Noble[a,*]

[a]*INSERM U705; CNRS UMR7157; Université Paris Descartes; Neuropsychopharmacologie des Addictions, 4 avenue de l'Observatoire-75270 PARIS Cedex, France*
[b]*IRIBHN, Université Libre de Bruxelles, 808, Route de Lennik, B-1070 Bruxelles, Belgium*

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Abstract

Behavioral and biochemical studies have suggested a functional link between the endogenous cannabinoid and opioid systems. Different hypotheses have been proposed to explain the interactions between opioid and cannabinoid systems such as a common pathway stimulating the dopaminergic system, a facilitation of signal-transduction- and/or a cannabinoid-induced enhancement of opioid peptide release. However, at this time, all the studies have been performed with exogenous agonists (delta-9-tetrahydrocannabinol or morphine), leading to a generally excessive stimulation of receptors normally stimulated by endogenous effectors (anandamide or opioid peptides) in various brain structures. To overcome this problem, we have measured various behavioral responses induced by the stimulation of the endogenous opioid system using the dual inhibitor of enkephalin-degrading enzymes, RB101, in CB1 receptor knockout mice. Thus, analgesia, locomotor activity, anxiety and antidepressant-like effects were measured after RB101 administration (80 and 120 mg/kg i.p. or 10 mg/kg, i.v.) in CB1 receptor knockout mice and their wild-type littermates. In all the experiments, inhibition of enkephalin catabolism produced similar modifications in behavior observed in CB1 knockout and wild-type mice. These results suggest limited physiological interaction between cannabinoid and opioid systems.

Theme: Neural basis of behavior
Topic: Neuropeptides and behavior

Keywords: Analgesia; Locomotor activity; Anxiety; Antidepressant-like effect; Knockout mice

1. Introduction

Cannabinoids and opioids are distinct drug classes that share similar pharmacological and biochemical profiles. Both compounds induce antinociception, reward effects and modulate emotional responses through activation of different receptors widely distributed in brain (mu, delta and kappa opioid and CB1 cannabinoid receptors), belonging to the superfamily of G-protein-coupled receptors (review in [26,37]). The existence of functional interactions between both systems has been suggested [8,20,44] that could be explain by common pathway stimulating the dopaminergic system [39], a facilitation of signal transduction [3] and/or an enhancement of opioid peptides released by cannabinoids [42]. In agreement with this latter hypothesis, a modulation of the endogenous opioid system through the CB1 cannabinoid receptors has been demonstrated. Thus, acute administration of THC and other cannabinoid agonists raised extracellular levels of endogenous dynorphin in the spinal

Abbreviations: CMS, chronic unpredictable mild stress; FAAH, fatty acid amide hydrolase; RB101, {*N*-[(*R*,*S*)-2-benzyl-3[(*S*)(2-amino-4-methyl-thio)-butyldithio]-1-oxo-propyl]-L-phenyl-alanine benzyl ester}; THC, delta-9-tetrahydrocannabinol

* Corresponding author. Fax: +33 1 53 73 97 19.
E-mail address: florence.noble@univ-paris5.fr (F. Noble).

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cord [29,45]. Moreover, in the brain, we have previously shown by microdialysis that THC induced an increase of endogenous enkephalins in synaptic cleft [42]. Endocannabinoid could also play a role as the CB1 receptor antagonist SR141,716A was able to antagonize opioid effects [23,33,43]. However, there is no evidence suggesting a role of endocannabinoids on endogenous opioid peptides release. The CB1 receptor has been disrupted in mice by homogenous recombination [18]. These mutant mice constitute a unique tool to determine the physiological role of the endocannabinoid and its physiological interactions with other neurotransmitter systems.

The aim of this study was to investigate whether the enkephalin release in the central nervous system could be under the control of an endogenous cannabinoid tone. Thus, we have studied the effects of endogenous enkephalins in CB1 knockout mice. However, the difficulty in this physiological approach is the rapid inactivation of these neuropeptides by metabolizing enzymes [34]. To overcome this problem, it is necessary to increase the life-time of the endogenous opioid peptide enkephalins. With this aim, we have used the systemically active inhibitor RB 101. This compound increases the synaptically released levels of endogenous enkephalins [11] without modifications in the peptide secretion [5]. In these conditions, only the responses submitted to a tonic or phasic control by endogenous enkephalins in particular brain structures are measured. As both opioids and cannabinoids are well known to modulate emotional-like responses, locomotor activity and pain perception, these responses were specifically evaluated.

2. Materials and methods

2.1. Animals

Animals CB1+/+ and CB1−/− were kept under standard animal housing conditions in a 12 h light–dark cycle (7 h 30 min:19 h 30 min) and maintained in a temperature-controlled room (21 ± 1 °C). Food and water were available ad libitum. CB1 null mutant mice were generated by homologous recombination as previously described [18]. Heterozygous mice were bred for 20 generations on a CD1 background before generating the wild-type and CB1 null mice used to produce the mice that have been used for the experiments. Adult female and male wild-type and CB1 null mice weighing 25–30 g at the beginning of the experiment were used. Estrus phase in females was not controlled, but no significant gender differences were observed in the different behavior tests used, in both wild-type and knockout animals (data not shown). Considering this observation, an equal number of males and females were used in each experimental group. Behavioral tests and care of the animals were in accordance with guidelines of the European Communities directive 86/609/EEC and under control of the local ethical committee.

2.2. Chemicals and drug administration

RB 101 {*N*-[(*R*,*S*)-2-benzyl-3[(*S*)(2-amino-4-methyl-thio)-butyldithio]-1-oxo-propyl]-L-phenyl-alanine benzyl ester} was synthesized in the laboratory as described previously [15] and was dissolved in the following vehicle: 10% EtOH, 10% cremophor EL (Sigma, France) and 80% H_2O. The intraperitoneal (i.p.) route was preferably used. However, when no significant responses were obtained at the higher dose of RB 101 that can be used (120 mg/kg), due to the low solubility of this compound, we used the intravenous (i.v.) route, which allows to get significant responses. The doses used (i.p. or i.v.) were chosen in agreement with previous studies [25,27]. RB 101 and control vehicle were administered in a volume of 0.1 ml/10 g, 10 min (i.v.) or 20 min (i.p.) before the behavioral tests. These times were selected on the basis of previous reports using the same compound, which correspond to the time of maximum effects [25].

2.3. Behavioral experiments

2.3.1. Locomotor activity

The total locomotor activity was measured in transparent activity boxes (19 × 11 × 14 cm). Horizontal displacements were determined by photocell beams located across the long axis and above the floor. Mice received RB101 (10 mg/kg, i.v.) or vehicle injection, and their locomotor activity was immediately recorded for 45 min, corresponding to the duration of action of RB 101 following i.v. administration [25]. Locomotor activity was expressed in scores (mean ± SEM) as the total number of interruption of the photocell beams. In order to habituate animals to the test environment and to obtain a stable baseline, they were placed in the activity boxes, during 1 h, two consecutive days before the test.

2.3.2. Hot plate test

A glass cylinder was used to keep the mouse on the heated surface of the plate, which was maintained at 52 ± 1 °C by a thermoregulated water-circulating pump [12]. The hot plate was performed 20 min after i.p. injection of vehicle or RB101 (80, 120 mg/kg, i.p.). The jumping responses were measured. The test latency is the time taken by the animal to jump. The cutoff was established at 240 s. In these conditions, no tissue damage to the paws occurred. Results were expressed as means ± SEM.

2.3.3. Forced swim test

The forced swim test was performed as described by Porsolt et al. [28] and Baamonde et al. [2]. Briefly, each mouse was placed during 6 min in a vertical Plexiglas cylinder (16 × 10 cm) with a water depth of 9 cm at 21–23 °C. The duration of the immobility, after a delay of 2 min, was measured during the last 4 min. The measurements were recorded after the RB101 was given at the dose of 80 and 120 mg/kg, i.p., 20 min before the assay.