Toxicology and Applied Pharmacology 286 (2015) 178–187

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Enhancement of endocannabinoid signaling protects against cocaine-induced neurotoxicity

Luciano R. Vilela [a], Pedro H. Gobira [b], Thercia G. Viana [b], Daniel C. Medeiros [c], Talita H. Ferreira-Vieira [c], Juliana G. Doria [a], Flávia Rodrigues [d], Daniele C. Aguiar [b], Grace S. Pereira [c], André R. Massessini [c], Fabíola M. Ribeiro [d], Antonio Carlos P. de Oliveira [b], Marcio F.D. Moraes [c,*,1], Fabricio A. Moreira [b,*,1]

[a] Graduate Program in Neuroscience, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil
[b] Department of Pharmacology, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil
[c] Department of Physiology, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil
[d] Department of Biochemistry and Immunology, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

ARTICLE INFO

Article history:
Received 9 February 2015
Revised 15 April 2015
Accepted 21 April 2015
Available online 29 April 2015

Keywords:
Endocannabinoid
Anandamide
FAAH
Cocaine
Seizures
PI3K

ABSTRACT

Cocaine is an addictive substance with a potential to cause deleterious effects in the brain. The strategies for treating its neurotoxicity, however, are limited. Evidence suggests that the endocannabinoid system exerts neuroprotective functions against various stimuli. Thus, we hypothesized that inhibition of fatty acid amide hydrolase (FAAH), the main enzyme responsible for terminating the actions of the endocannabinoid anandamide, reduces seizures and cell death in the hippocampus in a model of cocaine intoxication. Male Swiss mice received injections of endocannabinoid-related compounds followed by the lowest dose of cocaine that induces seizures, electroencephalographic activity and cell death in the hippocampus. The molecular mechanisms were studied in primary cell culture of this structure. The FAAH inhibitor, URB597, reduced cocaine-induced seizures and epileptiform electroencephalographic activity. The cannabinoid CB_1 receptor selective agonist, ACEA, mimicked these effects, whereas the antagonist, AM251, prevented them. URB597 also inhibited cocaine-induced activation and death of hippocampal neurons, both in animals and in primary cell culture. Finally, we investigated if the PI3K/Akt/ERK intracellular pathway, a cell surviving mechanism coupled to CB_1 receptor, mediated these neuroprotective effects. Accordingly, URB597 injection increased ERK and Akt phosphorylation in the hippocampus. Moreover, the neuroprotective effect of this compound was reversed by the PI3K inhibitor, LY294002. In conclusion, the pharmacological facilitation of the anandamide/CB1/PI3K signaling protects the brain against cocaine intoxication in experimental models. This strategy may be further explored in the development of treatments for drug-induced neurotoxicity.

Introduction

Addiction is a major public health problem with a high prevalence worldwide. Among the most commonly addictive drugs is cocaine, a psychostimulant and psychotomimetic compound (Degenhardt and Hall, 2012), which may induce acute and chronic psychiatric and neurological effects, including psychosis, agitation and seizures (Cadet et al., 2014). The molecular targets underlying cocaine-induced psychosis and behavioral stimulation include inhibition of neuronal monoamine uptake, particularly dopamine (Wise, 1984; Koob and Nestler, 1997). Accordingly, these effects are reversed by antipsychotic drugs, mainly through antagonism at dopamine D_2 receptor (Kishi et al., 2013). The mechanisms underlying the neurotoxic effects of this drug are less understood, since several intracellular biochemical processes seem to be involved (Cunha-Oliveira et al., 2008; Planeta et al., 2013). Thus, experimental models of cocaine intoxication are relevant for studying its biological mechanisms as well as new treatment approaches (Connors and Hoffman, 2013; Heard et al., 2011).

One possible strategy to reduce cocaine-induced neural damage would be facilitating protective mechanisms already at play in the brain. In this context, the endocannabinoid system has been proposed as an on-demand defense mechanism against neural hyper-excitability (Lutz, 2004; van der Stelt and Di Marzo, 2005; Fowler et al., 2010). This

Abbreviations: Akt, protein kinase B; CB_1, cannabinoid type-1 receptor; CB_2, cannabinoid type-2 receptor; D2, dopamine type-2 receptor; Δ^9-THC, Δ^9-tetrahydrocannabinol; EEG, electroencephalography; ERK, extracellular signal-regulated kinase; FAAH, fatty acid amide hydrolase; PI3K, phosphoinositide 3-kinase; TRPV1, transient receptor potential type-1 channel.

* Corresponding authors at: Institute of Biological Sciences, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, 31270-901, Belo Horizonte, MG, Brazil. Fax: +55 31 3409 2924.
E-mail addresses: mfdm@icb.ufmg.br (M.F.D. Moraes), fabriciomoreira@icb.ufmg.br (F.A. Moreira).
[1] These authors share senior authorship.

http://dx.doi.org/10.1016/j.taap.2015.04.013
0041-008X/

system is named after the *Cannabis sativa*, a plant whose main active constituent is Δ^9-tetrahydrocannabinol (Δ^9-THC). The typical components of the endocannabinoid system are the cannabinoid CB_1 and CB_2 receptors, their endogenous agonists (endocannabinoids), among which are arachidonoyl ethanolamide (anandamide) and 2-arachidonoyl glycerol; and the mechanisms mediating their synthesis and hydrolysis (Piomelli, 2003). The primary enzyme responsible for terminating anandamide actions is fatty acid amide hydrolase (FAAH) (Cravatt et al., 1996). Specific compounds have been developed to inhibit this enzyme and facilitate endocannabinoid signaling (Kathuria et al., 2003).

Cannabinoids and the endocannabinoid system have been investigated mainly in relation to their effects upon cocaine self-administration and psychomotor stimulation (Panlilio et al., 2010; Xi and Gardner, 2008; Moreira et al., 2015). Anandamide, however, is synthesized and release particularly in response to excessive neural activity, providing on-demand protection though CB_1 receptor signaling in the hippocampus (Marsicano et al., 2003; Monory et al., 2006). Moreover, compounds that inhibit anandamide hydrolysis induce neuroprotective effects (Karanian et al., 2007; Slusar et al., 2013) and attenuate seizures and electroencephalographic (EEG) activity induced by experimental convulsant substances (Karanian et al., 2007; Vilela et al., 2013, 2014). Thus, it is reasonable to suppose that CB_1 receptor activation also protects against cocaine toxicity. Indeed, one study observed that cannabinoids reduce seizures induced by various substances, including cocaine (Hayase et al., 2001). In spite of this evidence, the effects of anandamide hydrolysis inhibition upon cocaine neurotoxicity have not been investigated. Importantly, compounds acting through this mechanism facilitate the on-demand actions of anandamide and therefore have low propensity to induce motor impairment and other deleterious outcomes. This is in contrast to Δ^9-THC and other cannabinoids, which directly activate the CB_1 receptor and lack spatial and temporal resolution in their actions (Moreira and Wotjak, 2010).

The CB_1 receptor is coupled to diverse intracellular signaling pathways, including the phosphoinositide 3-kinase/protein kinase B/extracellular signal-regulated kinase (PI3K/Akt/ERK) pathway, which is known to exert neuroprotective functions (Galve-Roperh et al., 2002; Ozaita et al., 2007). Considering these pieces of evidence, we hypothesized that facilitating anandamide actions confers protection against cocaine excitotoxic through recruitment of the intracellular PI3K/Akt/ERK pathway in the hippocampus. Thus, we tested the effects of FAAH inhibition against seizures, EEG activity and hippocampal cell death in an experimental model of acute cocaine intoxication. To investigate the role of the CB_1 receptor/PI3K/Akt/ERk pathway, we performed experiments with selective compounds both in animals and in hippocampal cell culture.

Methods

Animals. Male Swiss mice (20–30 g) from the animal facility of the Federal University of Minas Gerais were kept on a 12 h:12 h dark/light cycle at 22 ± 1 °C with free access to food and water. All efforts were made to minimize animal suffering and to employ the least number of animals in each experiment. The protocols are in accordance with the Ethical Committee for Animal Experimentation (CETEA) of this University (Protocol 242/2013), which follows the Brazilian law no. 11.794/2008 and are in accordance with the international statement on the requirement for research involving animals (The Basel Declaration) and the Concordat on Openness on Animal Research.

Chemicals and injection protocols. The following compounds were used: The selective FAAH-inhibitor, [3-(3-carbamoylphenyl)phenyl]N-cyclohexylcarbamate (URB597); the CB_1 receptor selective agonist arachidonoyl chloroethylamide (ACEA); the CB_1 receptor antagonist/inverse agonist, 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-1-piperidinyl-1H-pyrazole-3 carboxamide (AM251). They were purchased from Cayman Chemical Company (1180 East Ellsworth Road, Ann Arbor, Michigan 48108, USA) and suspended in ethanol/cremophor (Sigma)/0.9% saline (1:1:18). Cocaine hydrochloride (Merck, Whitehouse Station, New Jersey, USA) was dissolved in saline; and the PI3K inhibitor 2-morpholin-4-yl-8-phenylchromen-4-one (LY294002; Tocris, Tocris House, IO Centre, Moorend Farm Ave, Bristol, Avon BS11 0QL, UK), was dissolved in DMSO 1% with a final concentration of 0.1%. All compounds have purity higher than 98%, as informed by each supplier. Rabbit anti-phospho Akt, anti-phospho ERK1/2, anti-Akt and anti-ERK1/2 monospecific clonal antibodies were from DB Biotech (Kosice, Slovakia). All injections were performed intraperitoneally (i.p.) in a volume of 1 ml kg^{-1}.

Surgery. The animals were anesthetized with ketamine (60 mg kg^{-1}) and xylazine (8 mg kg^{-1}) and positioned in a stereotaxic frame (David Kopf model 960). Stainless steel bone-screw electrodes (Fine Sciences Tools, mod. 19010-00) were placed over parietal cortices and fixed to the skull with zinc cement and soldered to pin bars. Coordinates (AP = 2.0 mm, ML = ± 2.0 mm referenced from the bregma suture) were derived from a stereotaxic atlas for mice (Paxinos and Watson, 1997). A reference electrode was inserted into the nasal bone. The animals received prophylactic intramuscular injections of polyantibiotic (0.27 g kg^{-1}; benzylpenicillin, streptomycin and dihydrostreptomycin Pentabiotico®, Fort Dodge, Brazil) and the nonsteroidal anti-inflammatory drug flunixinmeglumine (0.025 g kg^{-1}; Banamine®, Schering Plough, Brazil). They were allowed to recover for 4–5 days before the experiments.

Cocaine-induced seizures. For quantification of seizures, the animals received cocaine injections and were immediately placed in individual chambers for 10 min in an isolated room. The lowest seizure-inducing dose of cocaine was selected based on a dose–response curve. Seizure was defined as the occurrence of tail clonus with myoclonic jerks and wild jumping or convulsions with loss of righting reflex (Gasior et al., 1999; Vilela et al., 2013). An experimenter, unaware of the treatments, quantified the latency and duration of seizures. Vehicle or URB597 were administered 30 min before cocaine. The CB_1 receptor antagonist AM251 was administered 10 min before URB597.

Electroencephalografic recording and analysis. Video-EEG recordings were performed starting 1 min before cocaine administration until 30 s after the seizures onset. The EEG signal was amplified (5000×), filtered (1 Hz high pass and 500 Hz low pass) and digitized using an A/D converter set at a sampling rate of 1 kHz (Aisha4 Kananda® Ltda, Belo Horizonte, MG, Brazil); the data were stored in a personal computer. The records were analyzed off-line according to the latency to the onset and the duration of the epileptiform discharge (Vilela et al., 2013).

Fos immunohistochemistry. Two hours after cocaine administration, animals were anesthetized with an overdose of urethane and perfused transcardially with saline (200 nl) followed by 4% paraformaldehyde (PFA 4%) in 0.1 M phosphate buffer (150 ml, pH 7.4). Brains were removed and post fixed over 2 h in PFA 4% and stored for 36 h in 30% sucrose for cryoprotection. Coronal sections (40 μm) were obtained in a cryostat in triplicate for imunohystochemistry, as previously described (Beijamini and Guimarães, 2006). Briefly, tissue sections were washed with phosphate buffer in saline (PBS) and incubated overnight at room temperature with rabbit IgG in PBS (1:1000). The sections were washed in PBS and incubated with a biotinilated anti-rabbit IgG (1:1000). Fos immunoreactivity was revealed by the addition of the chromogen diaminobenzidin (DAB, from Sigma) and visualized as a brown precipitate inside the neuronal nuclei.

Cell death in the hippocampal tissue from cocaine-treated animals. The animals were killed by cervical displacement 24 h after cocaine injections. Their brains were rapidly (<1 min) removed and submerged in cold (2–3 °C) ACSF (artificial cerebrospinal fluid) solution: (in mM)