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Differential modulations of striatal tyrosine hydroxylase and dopamine metabolism by cannabinoid agonists as evidence for functional selectivity *in vivo*

Barbara Bosier [a], Giulio G. Muccioli [b], Birgit Mertens [c], Sophie Sarre [c], Yvette Michotte [c], Didier M. Lambert [d], Emmanuel Hermans [a,*]

[a] Neuropharmacology Group, Institute of Neuroscience, Université catholique de Louvain, 54.10, Av. Hippocrate 54, B-1200 Brussels, Belgium
[b] Bioanalysis and Pharmacology of Bioactive Lipids Laboratory, Louvain Drug Research Institute, Université catholique de Louvain, B-1200, Brussels, Belgium
[c] Department of Pharmaceutical Chemistry and Drug Analysis, Center for Neuroscience, Vrije Universiteit Brussel, 1090 Brussels, Belgium
[d] Pharmaceutical Chemistry and Radiopharmacy Group, Louvain Drug Research Institute, Université catholique de Louvain, B-1200 Brussels, Belgium

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ABSTRACT

It is generally assumed that cannabinoids induce transient modulations of dopamine transmission through indirect regulation of its release. However, we previously described a direct cannabinoid-mediated control of tyrosine hydroxylase (TH) expression, *in vitro*. We herein report on the influence of cannabinoid agonists on the expression of this key enzyme in catecholamine synthesis as well as on the modification of dopamine content in adult rats. As expected for cannabinoid agonists, the exposure to either Δ^9-THC, HU 210 or CP 55,940 induced both catalepsy and hypolocomotion. Supporting a possible long-lasting control on dopaminergic activity, we noticed a significant HU 210-mediated increase in TH expression in the striatum that was concomitant with an increase in striatal dopamine content. Surprisingly, while a similar trend was reported with Δ^9-THC, CP 55,940 completely failed to modulate TH expression or dopamine content. Nevertheless, the access of CP 55,940 to brain structures was validated by determinations of drug concentrations in the tissue and by *ex vivo* binding experiments. Furthermore, confirming the central activity of CP 55,940, the analysis of dopamine metabolites revealed a reduction in striatal DOPAC concentrations. Consistent with the involvement of the CB_1 cannabinoid receptor in these different responses, both HU 210- and CP 55,940-mediated effects were prevented by SR 141716A. Therefore, the present data suggest that both HU 210 and CP 55,940 cause a delayed/persistent regulation of the dopamine neurotransmission system. Nevertheless, these commonly used cannabinoid agonists endowed with similar pharmacodynamic properties clearly triggered distinct biochemical responses highlighting the existence of functional selectivity *in vivo*.

1. Introduction

While G protein coupled receptors (GPCRs) were initially considered as simple molecular switches, the last decade has revealed the complexity and flexibility offered by these membrane receptors. Indeed, experimental data, evidencing that a single GPCR has the ability to simultaneously activate multiple G protein subtypes, have accumulated for almost all members of this receptor family. Assuming that these different G protein couplings emerge from various active receptor conformations, this concept supports the possibility of agonist-selective signalling. This concept, also referred to as "agonist trafficking of receptor signalling" (Kenakin, 1995), has been more recently termed functional selectivity (Urban et al., 2007), which is suggestive of the potential selective regulation of functional responses.

With respect to the CB_1 cannabinoid receptor, its interaction with several G protein subtypes is commonly documented (for review see Hudson et al., 2010). This is consistent with accumulating reports showing agonist-selective activations of different G protein subtypes and associated signallings (Bonhaus et al., 1998; Glass and Northup, 1999; Lauckner et al., 2005; Mukhopadhyay and

Abbreviations: CP 55,940, (*1R,3R,4R*)-3-[2-hydroxy-4-(1,1-dimethylheptyl) phenyl]-4-(3-hydroxypropyl)cyclohexan-1-ol; DMSO, dimethylsulfoxide; DOPAC, 3,4-dihydroxyphenylacetic acid; DSE, depolarisation-induced suppression of excitation; DSI, depolarisation-induced suppression of inhibition; GPCR, G protein coupled receptor; HU 210, (6*R*)-*trans*-3-(1,1,-dimethylheptyl)-6,7,10,10-tetrahydro-l-hydroxy-6,6-dimethyl-6*H*-dibenzo[b,d]pyran-9-methanol; SR 141716A, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*1H*-pyrazole-3-carboxamide hydrochloride; Δ^9-THC, Δ^9-tetrahydrocannabinol; TH, tyrosine hydroxylase.

* Corresponding author. Tel.: +32 2 7649339; fax: +32 2 7645460.
E-mail address: emmanuel.hermans@uclouvain.be (E. Hermans).

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Howlett, 2005). In a previous study, we demonstrated an opposite regulation of the dopamine synthesising enzyme tyrosine hydroxylase (TH) expression in response to either HU 210, a tricyclic synthetic cannabinoid agonist or CP 55,940, a bicyclic cannabinoid agonist, in a neuroblastoma cell line (Bosier et al., 2007). Because these agonist-selective responses were regulated through different signalling pathways, our data unveiled functional selectivity at the CB_1 cannabinoid receptor (Bosier et al., 2009). However, the question of the physiological consequences of such a complex regulation remains unanswered.

There is evidence for the implication of dopamine in the central actions of cannabinoids in rodents and humans (Fernandez-Ruiz et al., 2010). Indeed, CB_1 cannabinoid receptors are abundantly expressed in basal ganglia (Herkenham et al., 1991), including those regions implicated in the control of reward and motor behaviours which are considerably altered by cannabinoids. On the other hand, endocannabinoids are now emerging as key components in the regulation of dopamine neurotransmission (Maldonado et al., 2006). Thus, by increasing the firing rate of dopaminergic neurons, cannabinoids facilitate dopaminergic transmission in several brain regions including the striatum (Andre et al., 2010; Cheer et al., 2004; Riegel and Lupica, 2004). However, contradictory results have been reported regarding dopamine release in striatum (Cadogan et al., 1997; Malone and Taylor, 1999; O'Neill et al., 2009; Szabo et al., 1999). Indeed, in this structure, as in most of the brain, CB_1 cannabinoid receptors are primarily located on presynaptic GABAergic and glutamatergic nerve terminals where they function together with the endocannabinoids as a retrograde signalling system. Hence, it is generally assumed that modifications of dopaminergic circuits mediated by CB_1 cannabinoid receptors are exerted through the modulation of either inhibitory or excitatory inputs received by the dopaminergic neurons.

Contradictory data have been reported concerning the presence of CB_1 cannabinoid receptors on dopaminergic neurons and it is likely that CB_1 cannabinoid receptors may regulate dopamine transmission through distinct and complementary mechanisms. Cachope et al. (2007) have shown that endocannabinoids-evoked dopamine release occurs through a mechanism that does not involve disinhibition of dopaminergic varicosities. It was also suggested that Δ^9-tetrahydrocannabinol (Δ^9-THC) could influence dopamine transmission through modulation of catecholamine uptake (Poddar and Dewey, 1980; Sakurai-Yamashita et al., 1989), although these *in vitro* observations were not supported by *in vivo* studies (Cheer et al., 2004). Finally, it has been reported that either a prenatal exposure to Δ^9-THC (Bonnin et al., 1994) or a chronic treatment with the synthetic cannabinoid agonist WIN 55,212-2 (Page et al., 2007) induce changes in TH expression and/or activity.

While we previously demonstrated a cannabinoid-mediated transcriptional regulation of TH expression *in vitro*, suggesting that CB_1 cannabinoid receptors may control critical neuronal functions through a delayed and persistent control of dopamine brain levels, no evidence for such a direct and acute regulation has been reported *in vivo*. To strengthen the physiological relevance of our study, and to further investigate the mechanisms of cannabinoid actions in the striatum, we have now examined the regulations of TH expression, dopamine content as well as dopamine metabolism after a single administration of cannabinoid agonists in adult rats. The complex regulation of TH expression that we reported in neuroblastoma cells has revealed functional selectivity at the CB_1 cannabinoid receptor. Therefore, we herein investigated whether this concept could account for agonist-selective responses *in vivo*. Thus, by reporting differential regulations of both TH expression and dopamine metabolism in the rat striatum consecutively to either HU 210 or CP 55,940 administration, this study provides evidence for physiological consequences of functional selectivity.

2. Experimental procedures

2.1. Animals

Male Wistar rats, weighing 275–300 g at the beginning of the experiment, were from Charles River Laboratories (distributed by Iffa-Credo, Lyon, France). Animals were acclimatised in the housing facility from Vrije Universiteit Brussel in a controlled environment (12 h daylight cycle, temperature controlled room) during 1 week before starting the experiments. All experiments were approved by the local ethic committee and housing conditions were as specified by the Belgian Law of 14 November, 1993 on the protection of laboratory animals (LA 1230314).

2.2. Drugs

HU 210 and CP 55,940 were purchased from Tocris Cookson (Bristol, UK) and Δ^9-THC was from Lipomed (Arlesheim, Switzerland). The CB_1 cannabinoid receptor inverse agonist/antagonist SR 141716A was generously given by Dr. Barth, Sanofi-Synthélabo Research (Montpellier, France). HU 210 and CP 55,940 were prepared as stock solutions in ethanol at 20 mg/ml, stored as aliquots at −80 °C and administered i.p. in a volume of 1 ml/kg 1% ethanol, 1% Tween 80 saline solution. Δ^9-THC was stored in ethanol solution at 100 mg/ml, prepared in 5% ethanol, 2% Tween 80 saline solution and administered i.p. in a volume of 2 ml/kg. SR 141716A was prepared in 4% ethanol, 1% Tween 80 saline solution, and administered i.p. in a volume of 1 ml/kg. In tests involving agonists only, rats were given a single i.p. injection, while for tests combining agonists and the antagonist SR 141716A, the latter was administered 10 min before injection of the agonist.

2.3. Catalepsy and locomotion measurements

Catalepsy and locomotion measures were performed as previously described (Bosier et al., 2010). Briefly, rats were tested for catalepsy by the placement of both forelimbs over a thin metal bar fixed at 10 cm above the ground and timed for the latency to move one or both forelimbs. After catalepsy testing, the motor activities of the animals were recorded in an open field device (60 × 60 cm arena) equipped with a digital video tracking system. The total walking distance was recorded during a 5 min period and scored with the Noldus EthoVision video tracking system (Wageningen, the Netherlands).

2.4. RNA extraction and quantitative real-time PCR

At the indicated time points, animal were sacrificed, the striatum was immediately dissected and stored at −80 °C. Total RNA was then extracted using TriPure reagent (Roche Diagnostics, Manheim, Germany). First strand cDNA was generated from 1 μg total RNA using the iScript cDNA Synthesis kit (Bio-Rad, Nazareth, Belgium) according to the manufacturer's instructions. Real-time PCR amplifications of TH cDNA were carried out using the iCycler IQ™ multicolour real-time PCR detection system (Bio-Rad), in a total volume of 25 μl containing 10 ng cDNA template, 0.3 μM of the primers (forward, 5′-AGTCCAATGTCCTGGGAGAACT-3′; reverse, 5′-TTCACCTGAGCCGGACTGCT-3′) designed to exclude the detection of genomic DNA, and the IQ™ SYBR Green Supermix. PCR protocol was conducted using 45 cycles with an annealing temperature of 60 °C. The fluorescence was monitored at the end of each elongation step. For quantitative analysis, a relative standard curve was generated using the same amplification conditions, with dilutions of a mix of cDNA templates (from 100 to 0.39 ng). TH mRNA expression was normalised to the relative amplification of GAPDH mRNA. Quantification of mRNA in the samples was performed using the post-run data analysis software provided with the iCycler system.

2.5. Western blot analysis

40 μg of striatum protein extracts diluted in the appropriate amount of 5× loading buffer (250 mM Tris–HCl, 500 mM dithiotreitol, 10% SDS, 50% glycerol, 0.5% bromophenol blue, pH 6.8) to obtain 1× buffer were boiled for 5 min before separation on a 10% SDS-polyacrylamide gel. Proteins were then transferred to nitrocellulose membranes for immunodetection. Blots were blocked for 1 h with 5% non fat powdered milk in TTBS (20 mM Tris, 150 mM NaCl, 0.05% Tween 20, pH 7.6) and probed at 4 °C overnight in a 1:2500 dilution of rabbit anti-TH (Chemicon, Hampshire, UK) antibody. This was followed by thorough washings in TTBS and 1 h incubation with HRP-conjugated anti-rabbit (1:3000) (Chemicon) secondary antibody. Blots were revealed with Super Signal West Pico system (Pierce, Aalst, Belgium). After antibodies stripping (100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris–HCl, pH 6.7, 60 °C, 30 min) the TH expression was normalised by reprobing with an anti-actin antiserum (1:5000) (Abcam, Cambridge, UK). Relative amounts of protein were quantified by scanning densitometry using the software Image Master (Pharmacia Biotech Benelux, Roosendaal, The Netherlands).

2.6. HPLC-MS quantification of exogenous cannabinoids

Rat brains were homogenised in H_2O (5 ml), sonicated in an ice-bath for 5 min after which 2.5 ml of the solution were added to 10 ml of $CHCl_3$ containing 2 nmol of