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Cannabinoid receptor 1 (CB₁) is a novel negative regulator of keratinocyte mitochondrial function in human epidermis *ex vivo*

Departments of ¹Physiology, ²Anatomy, Histology and Embryology, ³Medical Chemistry and ⁴Immunology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary; ⁵Monasterium Laboratory, Münster, Germany; ⁶Institute of Physical Chemistry, TU Bergakademie, Freiberg, Germany; ⁷Centre for Dermatology Research, University of Manchester, Manchester, UK

E-mail: ralf.paus@manchester.ac.uk; biro.tamas@med.unideb.hu; olah.attila@med.unideb.hu

Introduction

Epidermally expressed cannabinoid receptor 1 (CB₁) exerts strong **anti-inflammatory** actions, but also **impairs keratinocyte differentiation**. CB₁ is expressed both in the cell membrane (cmCB₁) and the **mitochondria** (mtCB₁) of several cell types, where mtCB₁ **directly suppresses mitochondrial activity**. However, it is unknown whether this also occurs in keratinocytes. Given the central role of mitochondrial function in keratinocyte biology, including cell aging, we have asked in the current study whether CB₁ plays any role in the **regulation** of human **epidermal mitochondrial biology *ex vivo*** (full-thickness human skin organ culture [hSOC]) and ***in vitro*** (primary human epidermal keratinocytes [NHEK]).

Aim

To assess whether cmCB₁ and/or mtCB₁ regulate mitochondrial functions of human epidermal keratinocytes *ex vivo* and *in vitro*, and impact on epidermal differentiation and/or the intraepidermal inflammatory milieu.

Conclusions, perspectives

- ✓ ‘Tonic’ endocannabinoid signaling **suppresses** keratinocyte **mitochondrial activity** in human epidermis, even though pharmacological CB₁ modulation does not significantly alter mitochondrial mass
- ✓ CB₁ activation **increases** epidermal **heat** production, and **decreases O₂ consumption** of human epidermal keratinocytes
- ✓ Human **epidermal keratinocytes express** both functional cmCB₁ and mtCB₁, and it is possible to pharmacologically differentiate between the functional activities of these receptor populations
- ✓ **Anti-inflammatory** actions of CB₁ may be mediated by cmCB₁, whereas **differentiation-impairing** effects could be coupled to mtCB₁ signaling

Most importantly, these findings introduce mtCB₁ as a novel, negative regulator of epidermal mitochondrial activity, which may impair keratinocyte differentiation.

Results

Abbreviations & glossary

ACEA: arachidonyl-2-chloroethylamide (cell penetrating CB₁ agonist; 30 μM)
AEA: anandamide (cell penetrating endocannabinoid, cannabinoid receptor agonist; 1 μM)
AM251: cell penetrating CB₁ inverse agonist (hSOC experiments: 1 μM; NHEK experiments: 4 μM)
Antimycin A: inhibitor of respiratory complex III (10 μg/ml; positive control for mtROS measurement)
Diff.: vehicle-treated differentiated keratinocyte culture
FAAH: fatty acid amide hydrolase (the most important endocannabinoid degrading enzyme)
Hemopressin: CB₁ inverse agonist (extracellularly restricted; 10 μM)
IL: interleukin
MTCO1: mitochondrially encoded cytochrome C oxidase I (member of respiratory complex IV)
NDUFS4: NADH:ubiquinone oxidoreductase subunit S4 (member of respiratory complex I)
PMA: phorbol-12-miristate-13-acetate (activator of classical and novel protein kinase C isoforms; 16 nM)
TFAM: mitochondrial transcription factor A (a master regulator of mtDNA synthesis)
UCP1: uncoupling protein 1
URB597: FAAH-inhibitor (it inhibits endocannabinoid degradation; 100 nM)
VDAC1: voltage-dependent anion channel 1 (histological marker of mitochondrial mass)

Activity of respiratory complex I (*in situ* nitroblue tetrazolium [NBT]-assay)

Figure 1 **Abrogation** of homeostatic **CB₁ signaling** by the CB₁-selective inverse agonist AM251 CB₁-dependently **increases** epidermal **activity** of respiratory complex I

Activity of respiratory complexes II-IV (*in situ* enzyme activity assay)

Figure 2 **AM251** CB₁-dependently **increases** epidermal **activity** of respiratory complexes II and IV

Epidermal heat production and UCP1 expression

Figure 3 Activation of **CB₁** **increases** epidermal **heat** production (chip-calorimetry of human skin), likely in a **UCP1-independent** manner

VDAC1 and TFAM expression (~effects on mitochondrial mass)

Figure 4 Modulation of CB₁ activity does not influence VDAC1 or TFAM expression in the epidermis, arguing for that **CB₁** is more **likely to directly regulate** mitochondrial **activity** of keratinocytes than influencing mitochondrial mass

MTCO1 expression (~expression of respiratory complex IV members)

Figure 5 AM251 slightly down-regulates MTCO1, suggesting that **CB₁** **may control** **activity of pre-existing respiratory complexes**, rather than expression of their components (NDUFS4 expression was unaffected, data not shown)

O₂ consumption of NHEKs

Figure 6 **CB₁** specific agonist ACEA **suppresses oxygen consumption** of NHEKs

Inflammatory response to stimulation with poly(I:C) (Toll-like receptor 3 activator, 20 μg/ml)

Figure 7 CB₁ is expressed **both** in the cell membrane (cmCB₁), and in the mitochondria (mtCB₁) of **human epidermal keratinocytes** (mitochondria are highlighted by red false color)

Mitochondrial CB₁ expression (immunoelectron microscopy)

Figure 8 Activation of **mtCB₁** **slightly increases mtROS** production in NHEKs

Mitochondrial ROS production of NHEKs

Figure 9 **Preliminary data** suggest that cmCB₁ and mtCB₁ may play **differential roles** in regulating human, epidermal keratinocyte biology; **anti-inflammatory** actions of CB₁ are likely to be mediated by cmCB₁, whereas **differentiation-impairing** effects are most probably coupled to mtCB₁

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