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Pharmacological characterization of rat VD-hemopressin(α), an α-hemoglobin-derived peptide exhibiting cannabinoid agonist-like effects in mice

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ABSTRACT

Hemopressin and related peptides have shown to function as the endogenous ligands or the regulator of cannabinoid receptors. Moreover, hemopressin and its truncated peptides were also reported to produce a slight modulatory effect on opioid system. In the present work, based on the amino acid sequence analyses of hemoglobin subunit α, rat VD-hemopressin(α) [(r)VD-Hpα] was predicted as a cannabinoid peptide derived from rat α-hemoglobin. Furthermore, (r)VD-Hpα was synthesized and characterized in a series of in vitro and in vivo assays. Our results demonstrated that (r)VD-Hpα induced neurite outgrowth in Neuro 2A cells via CB_1 receptor. In the tail-flick assay, (r)VD-Hpα dose-dependently exerted central antinociception through CB_1 receptor, but not CB_2 and opioid receptors. In mice, supraspinal administration of (r)VD-Hpα produced dose-dependent hypothermia, which was partially reduced by the CB_1 receptor antagonist AM251, but not by the antagonists of CB_2 and opioid receptors. In addition, (r)VD-Hpα caused hypoactivity after intracerebroventricular injection, and this effect was insensitive to the antagonists of cannabinoid and opioid receptors. Further assessment of the side-effects demonstrated that (r)VD-Hpα evoked the limited effects on gastrointestinal transit at antinociceptive doses, but repeated i.c.v. injection of (r)VD-Hpα induced development of antinociceptive tolerance. Taken together, these data suggest that the predicted peptide (r)VD-Hpα produces antinociception, hypothermia and hypoactivity via different pharmacological mechanisms, at least partially, which may offer an attractive strategy for separating cannabinoid analgesia from hypoactivity. Moreover, it implies that (r)VD-Hpα has therapeutic potential in pain management with limited side-effects.

1. Introduction

Cannabinoids have shown potent therapeutic roles in clinical trials and in animal models of various pain (Bushlin et al., 2010; Krustev et al., 2016; Pacher et al., 2006; Richardson, 2000). Cannabinoid receptors including CB_1 and CB_2 receptors are class A members of the G protein coupled receptor superfamily (Pertwee, 1997). The previous data demonstrated that CB_1 receptor was widely distributed in the central nervous system and peripheral tissues, while CB_2 receptor was mainly expressed in immune cells (Pacher et al., 2006; Pertwee, 1997, 2001). However, both CB_1 and CB_2 receptors were recently proved to play a critical role in pain modulation (Agarwal et al., 2007; Ibrahim et al., 2006; Pertwee, 2001).

Hemopressin and related peptides were identified as the novel ligands of cannabinoid receptors (Bomar and Galande, 2013; Gomes et al., 2009; Heimann et al., 2007). Hemopressin behaved as an inverse agonist or antagonist of the CB_1 receptor in vitro and in vivo (Dodd et al., 2010; Heimann et al., 2007). Firstly, hemopressin was reported as a novel bioactive peptide derived from hemoglobin, which could inhibit peripheral hyperalgesic responses in the inflammatory pain model, independently of opioid receptors (Dale et al., 2005a, 2005b). In addition, central or systemic administration of hemopressin caused antinociceptive actions, anorectic activities and anxiogenic-like effects (Bomar and Galande, 2013; Dodd et al., 2010; Fogaca et al., 2015; Heimann et al., 2007; Toniolo et al., 2014). However, hemopressin recently has shown to specifically activate CB_1 receptor in the

Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; CB_1, cannabinoid receptor type 1; CB_2, cannabinoid receptor type 2; CP55,940, (2)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol; dNPA, *D*.NP(*N*-Me)AFLFQPQRFamide; Hu-210, Δ^8-tetrahydrocannabinol dimethyl heptyl; i.c.v., intracerebroventricular; MPE, maximum percentage effect; PBS, Phosphate Buffer Solution; Δ^9-THC, Δ^9-tetrahydrocannabinol; (m)VD-Hpα, mouse VD-hemopressin(α); (r)VD-Hpα, rat VD-hemopressin(α); WIN55,212-2, (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-napthalenylmethanone.

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[^{35}S]GTPγS assays (Szlavicz et al., 2015). Furthermore, three hemopressin related peptides were identified as the endogenous agonists of cannabinoid receptors in vitro (Gomes et al., 2009). In addition, our recent data demonstrated that mouse VD-hemopressin(α) [(m)VD-Hpα, VDPVNFKLLSH-OH] was involved in pain modulation, cardiovascular action, food intake, locomotor activity, gastrointestinal and hypothermic modulation (Han et al., 2014; Li et al., 2014; Li et al., 2016; Pan et al., 2014). Central injection of (m)VD-Hpα produced antinociceptive effects in a CB_1 receptor-dependent manner (Han et al., 2014). Similar to the classical cannabinoid WIN55,212-2, (m)VD-Hpα induced antinociceptive tolerance after i.c.v. administration (Han et al., 2014; Pan et al., 2014). At the supraspinal level, (m)VD-Hpα also evoked hypothermia and hypoactivity in mice (Han et al., 2014). However, intrathecal injection of (m)VD-Hpα decreased arterial pressure via a non-CB_1 and non-CB_2 mechanism (Li et al., 2014).

A vast body of evidence suggests an important role of hemopressin and related peptides in the development of peptide-based pharmacological tools for the study of the endocannabinoid system (Bauer et al., 2012; Bomar and Galande, 2013; Dale et al., 2005b; Gomes et al., 2009; Heimann et al., 2007; Toniolo et al., 2014). The amino acid sequence of hemoglobin subunit α was reported to have apparent homology in different mammalian (Fig. 1). Based on these sequence analyses, we predicted that rat VD-hemopressin(α) [(r)VD-Hpα, VDPVNFK*F*LSH-OH] would be produced from α-chain of rat hemoglobin. It is worthy to note that hemopressin was obtained from α-chain of rat hemoglobin, while other hemopressin related peptides including (m)VD-Hpα were isolated from mouse brain. Thus, the C-terminal amino acid sequence of (r)VD-Hpα quite resembled that of hemopressin (PVNFKFLSH-OH).

To date, the reported data have shown that hemopressin and related peptides induced complex biological activities via different mechanisms, and the structure-activities relationship of these cannabinoid peptides remains unclear (Bauer et al., 2012; Dvoracsko et al., 2016; Gomes et al., 2009; Heimann et al., 2007; Szlavicz et al., 2015). In order to understand the structural and pharmacological properties of hemopressin and related peptides, different peptidic ligands of cannabinoid receptors will be further investigated. Recently, hemopressin and its truncated peptides were reported to produce a slight modulatory effect on opioid system (Szlavicz et al., 2015). In competitive receptor binding assays, both hemopressin and hemopressin(1–7) displayed a slight mu-opioid affinity by measuring in whole rat brain membrane homogenate and the CHO cells overexpressing mu-opioid receptors (Szlavicz et al., 2015). Moreover, [^{35}S]GTPγS binding assays also revealed the G-protein-stimulating effect of hemopressin in the cells expressing mu-opioid receptors (Szlavicz et al., 2015). Therefore, the present work was designed to study the total pharmacological profiles of the predicted peptide (r)VD-Hpα in a series of classical functional models. In addition, the role of cannabinoid and opioid receptors in (r)VD-Hpα-induced activities was also investigated by using the selective antagonists of CB_1, CB_2 and opioid receptors.

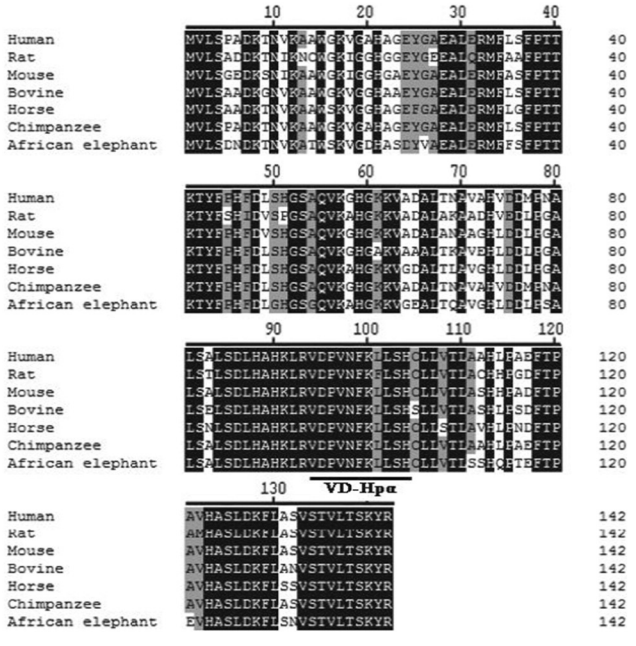

Fig. 1. Alignment of the peptide sequences of the hemoglobin subunit α of human, rat, mouse, bovine, horse, chimpanzee and African elephant. VD-hemopressin(α) (VD-Hpα) is underlined. The predicted peptide sequence of (r)VD-Hpα is VDPVNFKFLSH-OH. The NCBI Protein accession numbers are as follows: human hemoglobin subunit α, NP_000508; rat hemoglobin subunit α, NP_037228; mouse hemoglobin subunit α, NP_032244; bovine hemoglobin subunit α, NP_001070890; horse hemoglobin subunit α, NP_001078901; chimpanzee hemoglobin subunit α, NP_001036091; African elephant hemoglobin subunit α, NP_001267813.

2. Materials and methods

2.1. Drugs

The peptides including (r)VD-Hpα (VDPVNFKFLSH-OH) and (m)VD-Hpα (VDPVNFKLLSH-OH) were synthesized by manual solid-phase Fmoc (N-fluorenylmethoxycarbonyl) chemistry, as described previously (Han et al., 2014). The crude peptide was purified by preparative reversed-phase high-performance liquid chromatography using a C18 column as the solid. The identity of these peptides was confirmed using an electrospray ionization mass spectrometer (ESI-Q-TOF maXis-4G, Bruker Daltonics). The purity of the peptides was determined using analytical reversed-phase high-performance liquid chromatography.

In this study, WIN55,212-2, and naloxone were obtained from Sigma-Aldrich. The selective cannabinoid receptors antagonists AM251 and AM630 were purchased from Tocris. SR141716 were purchased from Cayman Chemical. Furthermore, in vitro, WIN55,212-2 and SR141716 were dissolved in 2% dimethyl sulfoxide/PBS. In in vivo studies, WIN55,212–2, AM251, AM630 and naloxone were dissolved in the vehicle (a 1:1:18 ratio of cremophor:dimethyl sulfoxide:saline solution). All other drugs were dissolved in sterilized saline, and the solutions were divided into aliquots and stored in 1.5 ml tubes at −20 °C. The aliquots were thawed and used on the day of the experiment.

2.2. In vitro neurite outgrowth assay

Neuro 2A cells were plated in 60 mm dishes and grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1‰ penicillin-streptomycin. In the neurite outgrowth assay, Neuro 2A cells were plated in 12-well plates and treated for 16 h in medium containing drugs as described previously (Gomes et al., 2009; Heimann et al., 2007; Jordan et al., 2005). Cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2. For each culture condition, randomly chosen regions of the plate containing 300 cells were scored under the microscope (Nikon TE2000-S). The cell having cellular projection of length at least two times longer than its diameter was considered neurite outgrowth of Neuro 2A cells.

2.3. Animals

Male Kunming (KM) mice (18–22 g) were provided by Animal Center of Lanzhou University and were housed in a climate-controlled room (22 ± 1 °C), with food and water available ad libitum. All animals were cared for and experiments were carried out in accordance with the European Community guidelines for the use of experimental animals (2010/63/EU). In addition, all the protocols in the present study were approved by the Ethics Committee of Lanzhou University, China. In the present behavior tests, each animal was used only once.

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