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Involvement of TRPV1 channels in the activity of the cannabinoid WIN 55,212-2 in an acute rat model of temporal lobe epilepsy

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ABSTRACT

The exogenous cannabinoid agonist WIN 55,212-2, (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo[1,2,3-de]-1,4-benzoxazin-6-Yl]-1-naphthalenylmethanone (WIN), has revealed to play a role on modulating the hyperexcitability phenomena in the hippocampus. Cannabinoid-mediated mechanisms of neuroprotection have recently been found to imply the modulation of transient receptor potential vanilloid 1 (TRPV1), a cationic channel subfamily that regulate synaptic excitation. In our study, we assessed the influence of pharmacological manipulation of TRPV1 function, alone and on WIN antiepileptic activity, in the Maximal Dentate Activation (MDA) acute model of temporal lobe epilepsy. Our results showed that the TRPV1 agonist, capsaicin, increased epileptic outcomes; whilst antagonizing TRPV1 with capsazepine exerts a protective role on paroxysmal discharge. When capsaicin is co-administered with WIN effective dose of 10 mg/kg is able to reduce its antiepileptic strength, especially on the triggering of MDA response. Accordingly, capsazepine at the protective dose of 2 mg/kg managed to potentiate WIN antiepileptic effects, when co-treated. Moreover, WIN subeffective dose of 5 mg/kg was turned into effective when capsazepine comes into play. This evidence suggests that systemic administration of TRPV1-active drugs influences electrically induced epilepsy, with a noticeable protective activity for capsazepine. Furthermore, results from the pharmacological interaction with WIN support an interplay between cannabinoid and TRPV1 signaling that could represent a promising approach for a future pharmacological strategy to challenge hyperexcitability-based diseases.

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Introduction

Epilepsy is broadly recognized as a multi-factorial neurologic disorder (Stafstrom and Carmant, 2015; Roseti et al., 2013), predominantly rooted on imbalanced excitability phenomena (Morelli et al., 2014). In this regard, endocannabinoids stand out for their major role in regulating synaptic function through various mechanisms (Di Marzo and De Petrocellis, 2012). Although cannabinoid (CB) transmission is principally known to act via a retrograde signaling pathway, novel evidence support the compelling importance of post-synaptic activation by cannabinoids of a particular class of ionotropic receptors, namely transient receptor potential vanilloid type 1 (TRPV1) (Castillo et al., 2012; Di Marzo and De Petrocellis, 2012; Yang et al., 2013; Wang et al., 2012). These ligand-gated, non-selective cation channels are expressed in critical regions of central nervous system (CNS) (Cristino et al., 2006; Menigoz and Boudes, 2011; Saffarzadeh et al., 2015), and augment the permeability to Na^+ and Ca^{++}, thereby modulating neuronal excitability (Szallasi and Blumberg, 2007). Putatively, TRPV1 are involved in mechanisms of synaptic efficiency such as LTP and LTD in the hippocampus, ultimately influencing pivotal processes of this brain region (Fu et al., 2009; Gibson et al., 2008; Leite et al., 2005). In support of the importance of CB non-retrograde modulation of TRPV1 conductances, immunocytochemical studies describe the post-synaptic co-localization of both CB receptor type 1 (CB_1) and TRPV1 in CNS synapses, hippocampus included (Cristino et al., 2006, 2008; Roberts et al., 2004; Castillo et al., 2012). As for hyperexcitability phenomena, vanilloid ligands have been suggested to make a relevant contribution to the regulation of epileptic activity, though their role is still controversial (Chen et al., 2013; Fu et al., 2009; Gonzalez-Reyes et al., 2013). For instance, the selective exogenous activator of TRPV1, capsaicin (CAP), was found to increase pathophysiological hippocampal firing, reverted by pretreatment with TRPV1 antagonist, capsazepine (CPZ) (Bhaskaran

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and Smith, 2010; Chen et al., 2013; Gonzalez-Reyes et al., 2013; Manna and Umathe, 2012). Consistently, recent data on human patients with mesial temporal lobe epilepsy (TLE) proved an increased TRPV1 expression in temporal cortex and hippocampus (Sun et al., 2013). In this context, reports about cannabinoids action on non-CB receptor targets brought knowledge that TRPV1 agonists such as anandamide (AEA, an endocannabinoid) or capsaicin, act on epileptic events and are challenged by CB_1 antagonist as well as by CPZ (Bhaskaran and Smith, 2010; Chavez et al., 2010; Di Marzo and De Petrocellis, 2012; Manna and Umathe, 2012). Noticeably, the majority of the cited studies on TRPV-active drugs are based on local injections *in vitro*, whilst research on systemic administrations on *in vivo* models remains limited. Taking this into consideration, our aim was to assess the outcomes of a pharmacological manipulation of TRPV1, with capsaicin and capsazepine, in an *in vivo* rat model of TLE, in the context of the reverberant hippocampal-parahippocampal circuitry (Sardo et al., 2006; Banach et al., 2011). Then, we pointed to evaluating the involvement of TRPV1 in cannabinoid effects applying a CB agonist, WIN 55,212-2 (hereafter WIN). To this purpose, we exploited an electrically induced acute model of epilepsy, the Maximal Dentate Activation (MDA) that was already employed to bring attention to the antiepileptic effect of WIN and the interplay of cannabinoids with other cellular signaling systems (Carletti et al., 2015; Rizzo et al., 2009, 2014). In this light, the current research falls within the remit of a more complex study about the unknown synaptic mechanisms through which endocannabinoids could exert neuroprotective effects, especially against hyperexcitability-based diseases.

Materials and methods

Animals and surgical procedures

The experiments were conducted in strict accordance with the Italian rules on animal experimentation and European directive (2010/63/EU).

Male Wistar rats (weight 260–300 g, 2–3 months old) were anaesthetized with urethane (1.2–1.4 g/kg intraperitoneally, i.p.), widely used for *in vivo* acute electrophysiological experiments (Hara and Harris, 2002; Maggi and Meli, 1986). The animals were positioned in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA) and the body temperature was maintained at 37–38 °C using a heating pad. Heart rate and pupil diameter were monitored during all the experimental session. Detailed surgical procedures have been described in our previous papers (Carletti et al., 2013). In brief, after craniotomy, both a stimulating electrode (coaxial bipolar stainless steel electrode: external diameter 0.5 mm; exposed tip 25–50 μm) and a glass recording electrode, (filled with 1% fast Green in 2 M NaCl) were stereotaxically placed (Paxinos and Watson, 1986), respectively, in the right angular bundle (AB: 1 mm anterior to the interaural line; 3–5 mm dorsal to it and 4.4 mm lateral to the midline) and in the ipsilateral dentate gyrus (DG: 6 mm anterior to the interaural line; 3.0 mm ventral to the cortical surface and 1.8 mm lateral to the midline). The animal was grounded through a subcutaneous Ag/AgCl wire in the scapular region.

Maximal dentate gyrus activation (MDA) and ictal events identification

The MDA protocol, originally used by Stringer and Lothman (1989) to trigger the activation of the DG, was modified as described in our previous research (Ferraro and Sardo, 2009; Sardo et al., 2009). In detail, fixed 10 s duration trains of 20 Hz stimuli was given through the AB stimulating electrode. Individual stimuli consisted of 0.3 ms biphasic pulses. The stimulus intensity was initially below that necessary to elicit any response and, then, it was increased in 100 μA steps until maximal dentate activation occurred (threshold intensity). Test stimulus trains were administered every 2 min until MDA appeared and then fixedly repeated every 10 min for up to 3 h. Furthermore, once the dentate activation was obtained, a stimulus intensity 100 μA higher than the intensity was used for the following stimulations to avoid desensitization phenomena. MDA was defined by a shift of the extracellular potential in DC-coupled recordings during stimulation time, as well as by the subsequent presence of bursts of population spikes of 20–40 mV. The DG bioelectric activity was recorded through a low level DC pre-amplifier (Grass 7B, West Warwick, RI, USA) and then processed by a software package provided by DataWave Technologies (Longmont, CO, USA). Then, the following MDA parameters were analyzed: (i) the time of onset (or latency) of the MDA was considered as the time from the beginning of AB stimulation to the midpoint of the DC potential shift; (ii) the total duration of the MDA was measured from the midpoint of the shift of the DC potential to the point at which the evoked paroxysmal activity abruptly ceased; and (iii) the after discharge (AD) duration was measured from the end of AB stimulation to the end of the epileptiform activity (Fig. 1A on the left). The electrophysiological parameters supply the following information about the effect of treatment during the experimental session: time of onset is relative to the susceptibility of the DG to respond to stimulation; MDA and AD durations are a measure that quantifies the extent of intra-stimulus and after-stimulus epileptic discharge. After achieving the MDA response, the eventual absence of it (Fig. 1A on the right) due to drug treatment during the experimental session was taken into consideration to describe the percentage of protection (% protection) against electrically induced epileptiform events. For each group, we calculated the % protection on the basis of the following formula: 100×(total number of no responses to stimulation per group/total number of stimulations per group).

Drug treatment

DMSO and WIN were purchased from Sigma Chemical Co. (Sigma, St. Louis, MO, USA), whereas capsaicin (CAP) and capsazepine (CPZ) were purchased from ABCam (Cambridge, UK). The study took into consideration 13 groups of rats ($n = 6$ rats each). The 1st (untreated controls) and 2nd (vehicle-treated) groups were studied for a period of about 200 min in order to verify possible modifications of MDA parameters due to the repetitive stimulations or to the vehicle administration. In the remaining groups the animals received CAP (10, 5 and 1 mg/kg, i.p., respectively per group), CPZ (2, 1 and 0.5 mg/kg, i.p.), WIN (10 and 5 mg/kg, i.p.), a co-treatment with CAP and WIN (both at 10 mg/kg, i.p; 11th group), a co-treatment with CPZ and WIN (2 mg/kg and 10 mg/kg, i.p, respectively; 12th group) and a co-administration with CPZ and WIN (2 mg/kg and 5 mg/kg, i.p, respectively; 13th group), at dosages and timing previously described (Carletti et al., 2015; Jia et al., 2015). All drugs were dissolved in the same final vehicle volume for each animal (15% of DMSO in saline solution). For all the experiments, each pharmacological treatment was performed only after five consecutive stable MDA responses (baseline period) and the subsequent observation period lasted 180 min after the drug injection (group treated with single drugs). In the last three groups, receiving two treatments each, due to different pharmacokinetic profiles of the drugs administered, an interval was interposed between administrations so as to allow coincident actions. In detail, the 11th group was pretreated with CAP 30 min before WIN injection, and the 12th and 13th groups were administered with CPZ 30 min before receiving treatment with WIN at 10 or 5 mg/kg. For all co-treated groups

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