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CANNABINOID AND NITRIC OXIDE SIGNALING INTERPLAY IN THE MODULATION OF HIPPOCAMPAL HYPEREXCITABILITY: STUDY ON ELECTROPHYSIOLOGICAL AND BEHAVIORAL MODELS OF TEMPORAL LOBE EPILEPSY IN THE RAT

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Abstract—A growing bulk of evidence suggests that cannabinoid system plays a pivotal role in the control of hyperexcitability phenomena. Notwithstanding, the anticonvulsant action of cannabinoids has not been fully addressed, in particular the involvement of potential cellular neuromodulators, for instance nitric oxide. In the current study, we focused on two distinct rat models of temporal lobe epilepsy, the Maximal Dentate Activation and the pilocarpine-induced acute seizures, providing both electrophysiological and behavioral data on cannabinoid and nitrergic system interplay. We evaluated the antiepileptic effects of 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), a CB agonist, and of 7-Nitroindazole (7NI), a preferential neuronal nitric oxide synthase (nNOS) inhibitor, at different doses, alone and in combination. MDA study showed that these drugs protected animals in a dose-dependent manner from electrically induced epileptiform discharges. In pilocarpine model, a dose-related activity of 7NI and WIN: (a) decreased the behavioral scoring, used to describe the severity of chemically induced acute seizures; (b) affected latency of the onset of acute convulsions; (c) dampened mortality rate. Interestingly, the combination of the treatments brought to light that individually ineffective doses of WIN turn into effective when nNOS activity is pharmacologically inhibited in both experimental conditions. This effect is mediated by CB_1 receptor since the co-administration of N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251), a CB_1 receptor specific antagonist, thwarted the 7NI–WIN convergent action. In the light of this, our findings suggest a putative antagonism between CBr-activated pathway and NO signaling in the context of neuronal hyperexcitability and contribute to elucidate possible synaptic processes underlying neuroprotective properties of cannabinoids, with a view to better integrate antiepileptic therapy. © 2015 Published by Elsevier Ltd. on behalf of IBRO.

Key words: hippocampus, temporal lobe epilepsy, cannabinoids, behavior, percentage of protection, electrophysiology.

INTRODUCTION

Epilepsy is a neurological disorder characterized by the appearance of spontaneously recurrent seizures (Hauser and Hesdorffer, 1990). In particular, temporal lobe epilepsy (TLE) is the most common type of partial complex seizure in adulthood (Hauser et al., 1996; Wieser, 2004), known for an unsatisfactory response to current therapy. Nowadays, many studies have been conducted on neuronal processes underlying epileptic states, particularly about the modulation of synaptic transmission. Over the last decades, growing interest has been rising on the role of cannabinoids (CB) as endogenous antiepileptic agents in the brain (Wallace et al., 2001; Hofmann and Frazier, 2013), despite the therapeutic application of their exogenous analogs may expose to acute and chronic side effects (Gerra et al., 2010; Hill et al., 2012). Cannabinoid receptors (CBr) contribute to the synaptic function modulating neurotransmitter signaling via a feedback mechanism since they couple to predominantly presynaptic Gi/o proteins (Howlett et al., 2002). In the hippocampus, the on-demand production of endocannabinoids from over-activated postsynaptic cells inhibits neurotransmitter release, hence protecting against excitotoxicity (Marsicano et al., 2003). Our previous experiments using the Maximal Dentate Activation (MDA) model of TLE revealed that (R)-(+) WIN 55,212-2 (hereafter, WIN), a CB non-selective agonist, exerts antiepileptic effects (Rizzo et al., 2009), in agreement with other authors (Wallace et al., 2001, 2003; Monory et al., 2006). Moreover, we highlighted that the endogenous cannabinoid receptor type 1 (CB_1) receptors, rather than

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Abbreviations: 7NI, 7-Nitroindazole; AB, angular bundle; AD, after discharge; AED, antiepileptic drugs; AM251, N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide; ANOVA, analysis of variance; CBr, cannabinoid receptors; DG, dentate gyrus; mAChr, muscarinic acetylcholine receptor; MDA, Maximal Dentate Activation; NMDAr, N-methyl-D-aspartate receptor; nNOS, neuronal nitric oxide synthase; SGC, soluble Guanylyl Cyclase; TLE, temporal lobe epilepsy; 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.

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type 2 (CB_2), may play a prominent role in WIN modulation of hyperexcitability (Rizzo et al., 2014). Notwithstanding, CB-mediated anticonvulsant effects in animal models are attributed not only to the functional involvement of the classical CB_1-dependent signaling (Jones et al., 2012; Hill et al., 2013; Payandemehr et al., 2015), but also to the interaction with further synaptic processes concerning modulatory messengers. Among these, nitric oxide (NO) has caught our attention since it apparently acts as a mediator for numerous cannabinoid effects (Kim et al., 2006a; Jones et al., 2008; Bahremand et al., 2009). This gaseous molecule exerts its effects mainly through activation of soluble Guanylyl Cyclase (sGC), a cGMP-producing enzyme (Feil and Kleppisch, 2008). As a matter of fact, general findings point to a co-localization of neuronal NO synthase (nNOS) and NO-activated sGC in synapses supplied with CB_1 receptors (Azad et al., 2001; Burette et al., 2002), particularly in the hippocampus (Makara et al., 2007), though the linkage between CB and NO systems in hyperexcitability phenomena still remains elusive. To this point, our laboratory yielded evidence of the influence of nNOS/sGC on MDA phenomena (Sardo et al., 2006) and on the modulation of therapeutic potential of antiepileptic drugs (AED) (Sardo et al., 2008, 2009; Ferraro and Sardo, 2009). As well, we outlined cannabinoid receptors and NO/sGC mutual implication in controlling electrically induced seizures (Rizzo et al., 2014), thus proposing an involvement of NO/cGMP-dependent pathway in the CB-activated responses.

It should be noted that studies about the pathophysiology of epileptic phenomena may produce conflicting results, for instance due to different experimental protocols, animal species and pharmacological properties of drugs chosen (Löscher et al., 1991; Löscher, 2011). For this reason, we designed a dual experimental approach to investigate on the cross-talk between CB and NO in the modulation of paroxysmal events, by applying the same treatments in two hippocampal *in vivo* models of TLE in the rat: the MDA and acute pilocarpine-induced seizures. These paradigms determine, respectively, electrically and chemically induced seizures, that resemble human refractory partial-complex epilepsy (Mello et al., 1993; Raza et al., 2001; Löscher, 2011). In the MDA, an excitatory re-entrant loop in the limbic system is activated by stimulation of the angular bundle (AB) (Stringer and Lothman, 1992). The pilocarpine protocol, based on the chemical triggering of cholinergic epileptogenesis in the hippocampus (Hamilton et al., 1997), causes spontaneous seizures with typical behavioral symptoms (Curia et al., 2008), poorly controlled by conventional AEDs (Glien et al., 2002; Chaki et al., 2006).

In the current research, WIN and 7-Nitroindazole (hereafter, 7NI), a preferential nNOS inhibitor, were initially assessed to cast new light on dose-related effects, in the MDA and, for the first time, to the best of our knowledge, in the acute pilocarpine protocol, where an implication of CB_1 receptors on WIN action was also inspected. Secondly, the co-treatment of these drugs was evaluated in a combination able to positively influence their possible interplay. Finally, 7NI–WIN cross-talk was assessed following the blockade of CB_1 receptors, using the selective antagonist AM251. This integrated experimental approach, drawing a parallel between TLE models with different epileptogenic mechanisms, could be helpful to place a more robust relationship between CB and NO neurotransmissions in the context of hippocampal hyperexcitability. Furthermore, investigating on the factors underpinning the neurobiological synaptic mechanisms of seizures may promote the development of novel non-conventional drugs.

EXPERIMENTAL PROCEDURES

Maximal Dentate Activation procedures

Male Wistar rats (weight 260–300 g, 2–3 months-old) were used in this study. Animal experiments were conducted in strict accordance with the European directive on animal experimentation (2010/63/EU). Detailed surgical procedures have been described in our previous paper (Carletti et al., 2013). Briefly: rats were anesthetized with urethane (1.2 g/kg intraperitoneally, i.p.) and after craniotomy, a stimulating electrode (coaxial bipolar stainless steel electrode: external diameter 0.5 mm; exposed tip 25–50 μm) was placed in the AB on the right side according to the stereotaxic coordinates of the Atlas of Paxinos and Watson (1986) (AB: 1 mm anterior to the interaural line; 3–5 mm dorsal to it and 4.4 mm lateral to the midline). A glass recording electrode, filled with 1% fast Green in 2 M NaCl, was placed in the ipsilateral dentate gyrus (DG) (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. The DG bioelectric activity was recorded through a low-level DC pre-amplifier (Grass 7B, West Warwick, RI, USA) and then processed by the SciWorks 5.0 package provided by DataWave Technologies (Longmont, CO, U.S.A.). According to our former studies (Carletti et al., 2013), we modified the protocol originally designed by Stringer and Lothman (1992), in order to obtain stable and reproducible MDA as well as to avoid progressive changes in its duration due to repetitive AB stimulations. Fixed duration (10 s) trains of 20-Hz stimuli were 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 it was increased by 100-μA steps in the following stimulations until MDA occurred (threshold intensity). The stimulus train was administered every 2 min until a MDA appeared and then every 10 min for up to 3 h. MDA was recorded by the electrode placed in the DG and it was defined by a shift of the extracellular potential in DC-coupled recordings as well as by the presence of bursts of population spikes (Fig. 1). Once the MDA was elicited, the percentage of responses to AB stimulation was analyzed to appraise the effect of pharmacological treatment; the eventual absence of MDA response per group was taken into consideration to evaluate the percentage of protection (% protection) against electrically induced epileptiform events on the

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