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Research report

CB1 receptor antagonism prevents long-term hyperexcitability after head injury by regulation of dynorphin-KOR system and mGluR5 in rat hippocampus

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ABSTRACT

Both endocannabinoids and dynorphin are feedback messengers in nervous system that act at the presynaptic nerve terminal to inhibit transmitter release. Many studies showed the cannabinoid–opioid cross-modulation in antinociception, hypothermia, sedation and reward. The aim of this study was to assess the influence of early application of cannabinoid type 1 (CB1) receptor antagonism SR141716A after brain injury on dynorphin-κ opioid receptor (KOR) system and the expression of metabotropic glutamate receptors (mGluRs) in a rat model of fluid percussion injury (FPI). Firstly, seizure latency induced by pentylenetetrazole was significantly prolonged 6 weeks after brain injury in group of SR141716A treatment. Then, PCR and western blot showed that SR141716A inhibited the long-term up-regulation of CB1 receptors in hippocampus. However, SR141716A resulted in long-term potentiation of dynorphin release and did not influence the up-regulation of KOR in hippocampus after brain injury. Furthermore, SR141716A reverse the overexpression of mGluR5 in the late stage of brain injury. We propose that during the induction of epileptogenesis after brain injury, early application of CB1 receptor antagonism could prevent long-term hyperexcitability by up-regulation of dynorphin-KOR system and prevention of mGluR5 induced epileptogenesis in hippocampus.

1. Introduction

Cannabinoid type 1 (CB1) receptors, mainly distributed in central nervous system, are located in both excitatory and inhibitory nerve terminals where they inhibit the release of glutamate orγ-aminobutyric acid (GABA) (Katona and Freund, 2008). CB1 receptors suppress neurotransmitter release when they bind endocannabinoids (eCBs) synthesized at the postsynapse in response to neuronal stimulation (Katona and Freund, 2008; Lutz and Monory, 2008). Several studies found CB1 receptor agonists are potent anticonvulsants (Monory et al., 2006; Wallace et al., 2003). Conversely, CB1 antagonists are proconvulsants in already hyperexcitable networks, such as in pilocarpine treated animals or tetanized tissue (Wallace et al., 2003; Chen et al., 2007). Unexpectedly, Echegoyen et al., (2009) showed rapid single application of CB1 receptor antagonist SR141716A following head injury prevented post-traumatic epileptogenesis. However, its mechanism of anti-epileptogenesis is unknown.

Increasing studies showed the importance of neuropeptides in epileptogenesis and epilepsy. There is evidence that neuropeptide dynorphin modulates neuronal excitability in hippocampus (Henriksen et al., 1982; Wagner et al., 1993; Weisskopf et al., 1993) and potentiates endogenous anti-ictal processes in human and animal models. Dynorphin exerts its actions mainly through κ-opioid receptors (KOR), which are located on presynaptical terminals and inhibits the release of neurotransmitters as CB1 receptors (Solbrig and Koob, 2004).

Many studies showed the cannabinoid–opioid cross-modulation in antinociception, hypothermia, sedation and reward (Robledo et al., 2008; Saez-Cassanelli et al., 2007). Previous studies

Abbreviations: CB, cannabinoid; KOR, κ opioid receptor; FPI, fluid percussion injury; PTZ, pentylenetetrazole; mGluRs, metabotropic glutamate receptors; GABA, γ-aminobutyric acid; eCBs, endocannabinoid; PTE, post-traumatic epilepsy

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have demonstrated that the necessity and sufficiency for activation of postsynaptic metabotropic glutamate receptors (mGluRs) in driving release of dynorphin and eCBs (Katona and Freund, 2008; Iremonger et al., 2011; Varma et al., 2001). Iremonger et al., (2011) found in their study dynamic interaction existed between these two retrograde messengers in vasopressin neurons. Specifically, bursts of presynaptic activity paired with postsynaptic spiking could cause the release of eCBs and dynorphin and inhibition of CB1 receptor could cause robust dynorphin release. Because dynorphin and endocannabinoid are co-expressed in hippocampus, especially in dentate gyrus (Solbrig and Koob, 2004; Loacker et al., 2007; Isokawa and Alger, 2005), thus we postulate that whether application of CB1 receptor antagonist after head injury could cause the long-term modulation of dynorphin-KOR system as well as mGluRs, which may function as one of anti-epileptogenesis mechanism for post-traumatic epilepsy (PTE).

2. Result

2.1. Effects of SR141716A on seizure latency and mortality in PTZ-induced seizure

Seizure latencies of different groups were evaluated by pentylenetetrazole (PTZ) injection 6 weeks after fluid percussion injury (FPI). Significant difference for myoclonic jerks onset latency was not found either between normal and FPI group (63.4 ± 8.0 vs. 55.7 ± 7.5 s, $p=0.266$) or between FPI+Veh and FPI+SR group (58.4 ± 4.8 vs. 64.3 ± 13.5 s, $p=0.382$). However, the FPI-induced decrease in clonic seizure onset latency was reversed by SR141716A (FPI group *vs.* normal group 61.8 ± 6.3 vs. 77.4 ± 5.7 s, $p<0.001$; FPI+SR vs. FPI+Veh: 77.3 ± 12.4 vs. 63.1 ± 5.0 s, $p<0.001$) (Fig.1). The rate of generalized tonic-clonic seizure (GTCS) onset and mortality in rat with PTZ decreased significantly after SR141716A application (GTCS FPI+Veh (n=10) *vs.* FPI+SR (n=10): 100% vs. 40%, *Fisher' Exact t Test* , $p=0.02$; Mortality FPI+Veh *vs.* FPI+SR: 90% vs. 30%, *Fisher' Exact t Test,* $p=0.011$).

2.2. Effect of SR141716A on the expression of CB1 receptor

Expression of CB1 receptor (Fig. 2A) demonstrated a trend of decrease instantly after FPI, with significantly lowest 24 h after FPI (Norm vs. FPI 24h: 1.07 ± 0.05 vs. 0.47 ± 0.07, $p<0.0001$). Then expression of CB1 receptor recovered and even higher than normal group 6 weeks after FPI (Norm vs. FPI 6w: 1.07 ± 0.05 vs. 3.13 ± 1.6, $p=0.007$). Western Blot of CB1 receptor did not show significant decrease at early stage after FPI (Norm vs. FPI 24h: 1.0 ± 0.0 vs. 1.23 ± 0.52, $p<0.05$). However, CB1 receptor increased significantly at late stage after FPI (Norm vs. FPI 6w: 1.00 ± 0.0 vs. 2.02 ± 0.42, $p<0.0001$) (Fig. 2B–C).

Results of polymerase chain reaction (PCR) (Fig. 3A) showed that application of SR141716A after FPI increased the expression of CB1 receptor significantly at 24 hours (FPI+SR vs. FPI+Veh 24 h: 2.9 ± 1.4 vs.0.47 ± 0.07, $p<0.001$). Six weeks after FPI, however, expression of CB1 receptor in SR141716A group was significantly lower than vehicle group (FPI+SR vs. FPI+Veh 6w: 1.21 ± 0.21vs. 3.31 ± 1.55, $p<0.01$). Western Blot of CB1 receptor did not show significant difference 24 h after FPI between SR141716A and vehicle group. However, CB1 receptor in SR141716A group 6 weeks after FPI was significantly lower than Vehicle group as PCR result (FPI+SR vs. FPI+Veh 6w: 1.19 ± 0.44 vs. 1.90 ± 0.48, $p<0.01$), with similar amount with normal group (Fig. 3B–C).

2.3. Effect of SR141716A on the expression of Dynorphin-KOR system

The expression of KOR showed a significant increase rapidly after FPI (sham vs. FPI 5 min: 1.05 ± 0.18 vs. 5.44 ± 1.42, $p<0.0001$), then maintained at a high level to 6 weeks (sham-FPI vs. FPI 6 weeks: 1.05 ± 0.18 vs. 5.32 ± 0.81, $p<0.0001$) (Fig. 4A). However, the expression of prodynorphin (precursor of dynorphin) decreased significantly right after FPI (sham-FPI vs. FPI 5 min: 1.03 ± 0.11 vs. 0.28 ± 0.14, $p<0.0001$), and maintained at a low level to 6 weeks after brain injury (sham-FPI vs. FPI 6 weeks: 1.03 ± 0.11 vs. 0.45 ± 0.09, $p<0.0001$) (Fig. 4B).

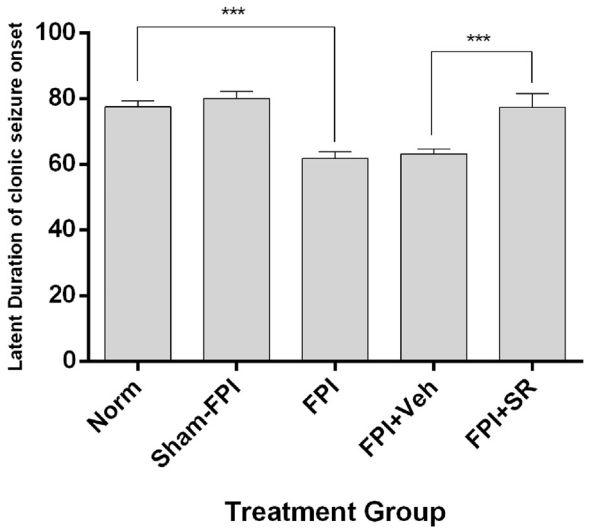

Fig. 1. Clonic **s**eizure onset latency of different groups in PTZ-induced seizure 6 weeks after FPI. FPI: fluid percussion injury, SR: SR141716A, Veh: vehicle; $^{***}p<0.001$.

SR141716A did not result in a significant change in the mRNA expression of KOR 6 weeks after FPI (FPI+Veh vs. FPI+SR 6 weeks: 5.32 ± 0.81 vs. 4.93 ± 0.91, $p>0.05$) (Fig. 5A). However, SR141716A caused a significant increase of predynorphin expression 6 weeks after FPI (FPI+Veh vs. FPI+SR 6 weeks: 0.43 ± 0.06 vs. 1.01 ± 0.17, $p<0.0001$) and recovered to level of sham-FPI group (Fig. 5B).

2.4. Effect of SR141716A on the expression of group I metabotropic glutamate receptors (mGluR type 1 and 5)

The expression of mGluR1 decreased significantly right after FPI and maintained at a low level to 6 weeks after brain injury (sham-FPI vs. FPI 6 weeks: 1.021 ± 0.15 vs. 0.24 ± 0.05, $p<0.0001$) (Fig. 6A). The expression of mGluR5 showed biphasic changes after FPI. Rapid significant increase was found 5 min after brain injury (sham-FPI vs. FPI 5 min: 1.01 ± 0.11 vs.1.38 ± 0.40, $p<0.05$) and then decreased to the lower level 1 week later (sham-FPI vs. FPI 1 week: 1.01 ± 0.11 vs. 0.53 ± 0.15, $p<0.0001$). However, the expression of mGluR5 showed a significant increase 6 weeks after FPI (sham-FPI vs. FPI 6 weeks: 1.01 ± 0.11 vs.7.01 ± 0.89, $p<0.0001$) (Fig. 6B).

Although SR141716A caused significant changes of the expression of mGluR1 at 1 week (FPI-Veh vs. FPI-SR 1 week: 0.13 ± 0.03 vs. 0.22 ± 0.10, $p<0.05$) and 6 weeks (FPI-Veh vs. FPI-SR 6 week: 0.24 ± 0.05 vs. 0.18 ± 0.03, $p<0.05$) after FPI, it did not reverse the low level of mGluR1 expression (Fig. 7A). On the contrary, SR141716A caused a significant increase of mGluR5 expression one week after FPI (FPI-Veh vs. FPI-SR 1 week: 0.65 ± 0.31 vs. 7.96 ± 1.03, $p<0.001$). However, the expression of mGluR5 after SR141716A treatment decreased significantly at 6th week compared with control group (FPI-Veh vs. FPI-SR 6 week: 7.01 ± 0.89 vs. 0.82 ± 0.17, $p<0.001$) (Fig. 7B).