Effects of chronic administration of PL017 and beta-funaltrexamine hydrochloride on susceptibility of kainic acid-induced seizures in rats

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Abstract: There is evidence that 5–7 d after acute seizure episodes induced by kainic acid (KA) the rats develop a long-lasting increase in the susceptibility to seizures followed by spontaneous recurrent seizures (SRS). The present study was focused on the role of hippocampal mu opioid receptors (MORs) in the susceptibility of rats to seizures with the KA model of epilepsy. The rats received a convulsant dose of KA (10 mg/kg, i.p.) were continuously infused with a selective MOR agonist PL017 (2.09, 2.59, 3.29 µg/µl), or a selective MOR antagonist β-funaltrexamine hydrochloride (β-FNA, 0.88, 1.10, and 1.35 µg/µl) into ventral hippocampus by means of mini-osmotic pumps. Seven days later, the susceptibility of rats to seizures was checked by a subconvulsant dose of KA (5 mg/kg, i.p.). PL017 infusion shortened the latency and increased the stage of seizures induced by subconvulsant dose of KA in a dose-dependent manner. In contrast, infusion of β-FNA exhibited a dose-dependent effect against seizures challenged by subconvulsant dose of KA. These results indicate that hippocampal MOR may exert a promoting effect on the susceptibility of rats to KA-induced seizures.

Key words: mu opioid receptor; susceptibility to seizures; hippocampus; epilepsy; kainic acid; rat

Epilepsy is a common brain disorder and characterized by spontaneous recurrent seizures (SRS). A single systemic injection of a convulsive dose of kainic acid (KA) results in both acute seizures (short-term effects) and chronic SRS (long-term effects) similar to human temporal lobe epilepsy[1,2]. This is widely used as a model of epilepsy. We demonstrated that 5–7 d after a single systemic injection of a convulsant dose of KA the rats developed a long-lasting increase in the susceptibility to seizures followed by SRS, therefore a subconvulsant (or
subthreshold) dose of KA, which had no convulsive effect when it was given alone, could induce seizures in these rats[31].

The formation of the susceptibility to seizures may be a key factor in SRS, but the mechanisms are not fully clear yet. Although it has been shown that KA-induced acute seizures are related to opioid peptide systems in the brain[40], few studies have evaluated the effects of opioid systems on the formation of the susceptibility to seizures after KA.

Activation of mu opioid receptors (MORs) in hippocampus has a net excitatory effect, which facilitates epileptogenesis[33]. Enkephalin (ENK) is a putative pro-convulsive opioid in hippocampus. ENK modifies KA-induced convulsions and behavioral effects predominantly by an action at MORs[41]. Injection of mu, but not delta or kappa, opioid receptor agonists can induce seizures[33]. KA injection induced an up-regulation of proenkephalin mRNA and its protein products in rat hippocampus[7]. Taken together, these results suggest that MORs may play an important role in the formation of the susceptibility to seizures. The present study was undertaken to determine the role of hippocampal MORs in the susceptibility to KA-induced seizures in rats with a selective MOR agonist PL017 (Tyr-Pro-N-MePhe-D-Pro-NH₂) or an irreversible MOR-selective antagonist β-FNA (β-funaltrexamine hydrochloride) given by a mini-osmotic pump.

1. MATERIALS AND METHODS

1.1 Animals. Adult male Sprague-Dawley rats weighing 180–220 g (Animal Center of Dalian Medical University, China) were housed under controlled conditions of temperature (21~23°C) and light (alternating 12-h light–dark cycle) with ad libitum access to standard rat food and tap water.

1.2 Groups. The rats were divided into normal control, saline infusion, PL017 infusion and β-FNA infusion groups to examine the effect of MORs on the formation of the susceptibility to KA-induced seizures. In PL017 and β-FNA infusion groups three different dosages were administered respectively to show the dose-dependent effects of them. Each group had 8–9 rats. KA, PL017 and β-FNA were purchased from Sigma Chemicals Co. (USA).

1.3 Preparation of mini-osmotic pumps. The ALZET model 2001 mini-osmotic pumps (USA) were used for intra-hippocampal injection. The pumps were filled with 200 μl PL017 (2.09, 2.59 and 3.29 μg/μl in saline, Sigma), β-FNA (0.88, 1.10 and 1.35 μg/μl in saline, Sigma), and 0.9% saline by a 0.5 ml syringe and the blunt-tipped 25-gauge filling tube. The flow moderators attached with catheters of PE60 size were filled by saline and inserted into the pumps. Brain infusion cannula, which had been sterilized in 70% ethanol, was attached with the other catheter’s head. The pumps were submerged in 37°C sterile saline over night to insure the pump system unobstructed.

1.4 Model establishment and animal treatment. For surgery, rats were anaesthetized with an intraperitoneal injection of 4% chloral hydrate (1 ml/100 g) and placed in the stereotactic frame (KOPF, USA) with the incisor bar set at 5 mm above the interaural line. The infusion cannulae were implanted in the left ventral hippocampus using stereotactic co-ordinates (5.8 mm posterior from the bregma, 4.6 mm lateral from the midline and 7.5 mm ventral from the outer surface of the skull according to the atlas of Paxion G and Watson C[33]), and then held in position by dental cement attached to two stainless steel screws driven into the skull. The pumps were inserted into a small pocket formed by spreading apart the subcutaneous connective tissues at scapular region. These model 2001 pumps can infuse drugs into selected nuclei in awake freely moving animals continually (7 d) and constantly (at mean pumping rate of 1.06 ±0.04 μl/h). KA (Sigma) was dissolved in 0.9% saline and adjusted to pH 7.0 with 2 mol/L sodium hydroxide and prepared the day before the experiment. 48 h after pumps implantation, a convulsive dose (10 mg/kg) of KA was injected subcutaneously in normal control, saline, PL017 and β-FNA groups respectively. 7 d later, all rats were given subcutaneously with a subconvulsant dose of KA (5 mg/kg) to determine the state of the susceptibility of rats to seizures induced by previous KA injection. The latency (the interval between injection of subconvulsant dose of KA and occurrence of first seizure), stage, and stage/time of behavioral seizures of different groups were recorded. The behavioral seizure was scored as described by Racine[39] (chewing, stage 1; head-nodding, stage 2; unilateral limbic clonus, stage 3; rearing with bilateral forelimb clonus, stage 4 and bilateral forelimb clonus with rearing and falling, stage 5). To verify the sites of the injection cannulae, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and perfused transcardially with 1% and 4% parafomaldehyde. Brains were removed, sectioned with a vibratome and stained with 0.1% thionin.

1.5 Statistical analysis. All results were expressed as mean ± standard error of the mean and analyzed using one-way ANOVA followed by post-hoc analysis with mul-
multiple comparisons. Difference was considered significant at $P<0.05$.

2. RESULTS

Subcutaneous injection of a convulsive dose of KA (10 mg/kg) made all rats express motor seizures over stage 4 within 60 min. After administration of subconvulsant dose of KA, the rats exhibited seizures different in latency and stage between different groups.

2.1 Effects of PL017 and β-FNA on the seizures

Mean latencies to first seizure after 5 mg/kg KA injection (Fig.1A) were $18.89\pm1.09$ min and $18.13\pm0.93$ min in the normal control and saline groups. Mean latency of the PL017 (3.29 µg/µl) group was $10.38\pm0.73$ min, shorter significantly than that of the normal control and saline groups ($P<0.05$). Mean latency of the β-FNA (1.35 µg/µl) group was $39.33\pm1.38$ min, which prolonged significantly compared with other groups ($P<0.01$). The mean seizure stages induced by subconvulsant dose of KA (Fig.1B) were $4.56\pm0.24$ in normal control group, $4.63\pm0.26$ in saline group, and $4.88\pm0.13$ in PL017 (3.29 µg/µl) group respectively, and there was no significant differences between them ($P>0.05$). The seizures in β-FNA (1.35 µg/µl) group occurred more weekly with a mean stage of $1.25\pm0.31$, which was reduced significantly compared with other three groups ($P<0.01$).

2.2 Time-course of seizure stage after PL017 or β-FNA treatment

The time-course of seizures caused by subconvulsant dose KA is shown in Fig.2. There was no difference between the normal control and saline groups ($P>0.05$). The seizure stages of the PL017 (3.29 µg/µl) group were more severe than those of the normal control and saline groups at different time points before they reached the maximal stage within 40 min ($P<0.01$). In the β-FNA (1.35 µg/µl) group, however, no seizures were found during the first 10 min. The seizure stages at all other time points were reduced significantly compared with the other three groups ($P<0.01$).

2.3 Dose-dependent effects of PL017 and β-FNA on the seizures

Figure 3A-D show the dose-dependent effects of PL017 and β-FNA. As shown in Fig. 3A and 3B, MOR agonist PL017 at doses of 2.09–3.29 µg/ml caused a decrease in the latency and an increase in the stages of subconvulsant dose KA-induced seizures in a dose-dependent manner. MOR antagonist β-FNA at doses of 0.88–1.35 µg/µl made the dose-dependent effects in the latency
and the stages of seizures reverse to PL017 (Fig. 3C and 3D).

3. DISCUSSION

The present study clearly demonstrates that the selective MOR agonist PL017 could accelerate and aggravate the seizures induced by subconvulsant doses of KA, but the MOR-selective antagonist β-FNA could retard and decrease the seizures challenged by subconvulsant dose of KA. These results indicate that the formation of the susceptibility to KA-induced seizures is mediated exclusively by MORs rather than delta or kappa receptors, which was the first direct evidence supporting the involvement of hippocampal MORs in the susceptibility to seizures in rats.

MORs are abundantly present in the hippocampus, especially concentrated in the dentate molecular layer of the ventral hippocampus[8]. Most MORs-labeled neurons possess the morphology and location of inhibitory interneurons and overlapping immunoreactivity for MOR and gamma-aminobutyric acid (GABA) has been observed in some hippocampal neurons[10]. Recent study has clearly showed that MORs are almost exclusively in GABAergic neurons, predominantly in their axons and axon terminals (presynaptic) in both hippocampus proper and dentate gyrus (DG)[6, 11]. As a main endogenous ligand of MORs, ENK is also present in several hippocampal formation locations[6, 10]. Ultrastructural work in the rats has showed that profiles with ENK-like immunoreactivity synapse directly onto GABAergic neurons[6, 10].

Activation of MORs has a net excitatory effect in hippocampal formation, and an increasing body of evidence suggests the important roles of MORs and ENK in the pathogenesis of epilepsy. Electrophysiological and behavioral studies suggest the involvement of MORs in the generation of limbic and absence seizure[5, 12, 13]. In GALR1 knockout mice that had been displaying seizures, a strong upregulation of ENK was found in the granule cells/mossy fibers[14]. Injection with selective agonists of MORs, but not of delta or kappa receptor produced seizures[15]. The upregulation of MORs could enhance the KA seizures[16]. KA could not only enhance the release of endogenous ligands and its affinity to MORs, but also produces significant increases in MORs number[4, 12]. These results indicate that the seizures were mediated by MORs rather...
than other opioid receptors. The excitatory effect of MORs is regionally specific. It’s shown that only injecting selective MOR agonist into the ventral hippocampus, but not into other brain regions such as the striatum, frontal cortex, amygdala, or dorsal hippocampus of rats, resulted in convulsions\cite{15}. These data suggest that MORs in ventral hippocampus may be a main proconvulsant target and the main origination of epileptic activity.

Although MORs may be responsible for the expression of seizures, it’s still not clear whether this type of receptor also plays a role in the SRS or the formation of the susceptibility to seizures. Our present study, by injecting the agonist or antagonist of MORs into ventral hippocampus, provided the first direct evidence that the MORs in ventral hippocampus mediated the formation of the susceptibility to KA-induced seizures.

Previous studies have demonstrated that MOR executes its excitatory effect through inhibition of GABA-containing interneurons\cite{10,17,18}. Although no direct evidence exists, it is possible that MORs mediate the formation of the susceptibility to seizures by means of GABA system as well. There is an abundance of MORs and GABA-ergic interneurons in the DG and hilus, which also are the main targets of the projections from ENK-containing perforant pathway (PP). The PP forms the major extrinsic source of hippocampal ENK and terminates mostly in the ventral hippocampus\cite{15}. It is known that ENK could inhibit the transmitter release of GABA-ergic interneurons by acting on the presynaptic MORs and result in the suppression of GABA-ergic synaptic transmission\cite{10,18}. Under normal condition, GABA-ergic interneurons mediate surrounding inhibition, so the subconvulsant dose of KA could not induce seizures when it was given alone. However, when GABA-ergic interneurons in hilus decreased in their number or functions after convulsant dose of KA, the surround inhibition in a confined area will be eroded (disinhibition). The resulting enlarged aggregate of hyperexcitable granule cells may then respond to the subconvulsant dose of KA with excessive discharges. DG has a high density of MORs and is also the region where ENK-containing PP fibers from the entorhinal cortex and interneuronal GABA-containing fibers terminate. KA could up-regulate the expression of PENKmRNA in hippocampus continuously. So the increased ENK persistently inhibits GABA transmitter release by combining with MORs on GABA-ergic neurons and then remove the surrounding inhibition, inducing a persistent high-excitability in hippocampal pathway and resulting in the formation of the susceptibility to seizures. The epileptiform activity in hippocampus, when radiated to the motor area of the brain, may lead to the expression of behavioral SRS. This may be a key mechanism for participation of MORs in the formation of the susceptibility of rats to KA-induced seizures.

Results from the present study showed that MORs in hippocampus promote the formation of the susceptibility of rats to KA-induced seizures, indicating mu opioid system play a role not only in acute seizures, but also in the development and maintenance of SRS. This suggests a possibility that the mu opioid system may be a potential target for the development of antiepileptic drugs.

REFERENCES


