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The effect of selective opioid receptor agonists and antagonists on epileptiform activity in morphine-dependent infant mice hippocampal slices

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ABSTRACT

Hippocampal slices of mouse brain were used to estimate how selective agonist and antagonist of opioid receptors alter Low-Mg^{+2} artificial cerebrospinal fluid (LM-ACSF)-induced epileptiform activities in normal and morphine-dependent mice. Brain slices were obtained from control and morphine-dependent mice. The morphine-dependent group received morphine once a day for 5 consecutive days, and the control group received saline. All injections were administered subcutaneously (s.c) in a volume of 0.1 mL on postnatal days 14–18. Brain slices were perfused with LM-ACSF along with selective agonist and antagonist of μ, κ and δ opioid receptors. Changes in spike count per unit of time were used as indices to quantify the effects of LM-ACSF exposure in the slices. In both groups, DAMGO (selective μ opioid receptor agonist) and DPDPE (selective δ opioid receptor agonist) suppressed while Dyn-A (selective κ opioid receptor agonist) potentiated the epileptiform activity. Meanwhile, BFN-A (selective μ opioid receptor antagonist) recovered epileptiform activity in normal brain slices but not in morphine-dependent ones. NTI (selective δ opioid receptor antagonist) and nor-BNI (selective κ opioid receptor antagonist) decreased epileptiform activity. It seems that the excitatory effect of morphine on epileptiform activity was mediated through kappa receptors and its inhibitory effect was mediated via the mu receptor and, to a lesser degree, through the delta receptor. The pattern of effect was similar in normal and morphine-dependent slices, but the intensity of the effect was significantly stronger in normal mice. Finding of this study might be considered for further research and attention in epilepsy treatment.

1. Introduction

Early-life seizures have been associated with later cognitive dysfunction, depending on their type, duration, and frequency as well as the age of onset (Mikroulis and Psarropoulou, 2012). Epilepsy is a common neurological disorder affecting over 1% of the adult population (Hauser and Hesdorffer, 1990) and is associated with long-lasting neuronal plasticity changes that have been well documented in humans and replicated in a variety of animal models (Scharfman, 2002; Spencer, 2002). Because epileptic brain tissue is unique and manifests long-lasting alterations, it has been recommended that models of epileptic tissue be employed to test anticonvulsant agents and study the mechanisms of epileptogenesis (Stables et al., 2002). Opioids appear to have a complex influence on neuronal excitability. The pro- or anti-convulsant activity of an opioid compound depends not only on its affinity to specific opioid receptor, but also on the dose, route of administration, and animal model (Saboory et al., 2007; Saboory et al., 2014). Generally, opioids tend to inhibit neuronal activity. The stimulation of postsynaptic μ opioid receptors (MORs) activates potassium channels and inhibits voltage-dependent calcium channels, leading to hyperpolarization. Accordingly, some early studies showed that morphine suppresses audiogenic seizures and delays the onset of pentetrazole- and flurothyl-induced convulsions (Frenk et al., 1979; Thompson et al., 2009). When doses which are much higher than those producing analgesia are used, morphine and related opiates may produce convulsions (Frenk, 1983; Frenk et al., 1982). Meanwhile, morphine-pretreated animals show a faster acquisition of seizure activity (Hofmann et al., 2006). It has also been reported that early exposure to chronic morphine in infant rats might change their susceptibility to PTZ-induced seizure in an age-dependent manner

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(Gholami and Saboory, 2013; Gholami et al., 2012). The mechanism of opioid-induced epileptic activity was partially elucidated. For instance, it has been hypothesized that opioids acting on MOR inhibit the hippocampal GABA release, causing the disinhibition of pyramidal cells and epileptic activity. Indeed, an immunohistochemical study has confirmed that MORs are localized mainly on hippocampal interneurons, innervating pyramidal cell distal dendrites (Drake and Milner, 2002). In contrast, prodynorphin peptides released from granule cells during seizures decrease excitatory transmission by activating presynaptic κ opioid receptors (KORs) on perforant path neuronal terminals (Simmons et al., 1994). Taking into account the hyperpolarizing effect of opioids in a majority of brain regions except for the hippocampus, it has been postulated that endogenous opioid systems may play a crucial role in the self-limitation of seizures (Frenk et al., 1979; Velisek and Mares, 1992). It was found that DAMGO – a selective agonist of MOR – enhances absence epilepsy in rats, whereas the δ opioid receptor (DOR) agonist (DPDPE) showed no such effect. Interestingly, KOR agonists suppressed absence seizures in rats (Przewłocka et al., 1995). Some new facts confirm the putative anticonvulsant effects of dynorphin and other KOR agonists in self-sustaining status epilepticus in rats (Mazarati and Wasterlain, 2002) and ethanol withdrawal seizures in mice (Beadles-Bohling and Wiren, 2006). Other studies reported somewhat different roles for opioid receptors in various models of seizures (Feng et al., 2012; Saboory et al., 2007). Moreover, the differential effect of these receptors on seizure is not clear in normal and morphine-dependent subjects, particularly in developing brain. The purpose of this study was to evaluate the probable pro-seizure and/or anti-seizure effects of selective agonist and antagonist of opioid receptors on experimental epileptiform activity induced by LM-ACSF on hippocampal slices of normal and morphine-dependent infant mice.

2. Materials and methods

Ingredients for the preparation of artificial cerebrospinal fluid, such as NaCl, NaH_2PO_4, $NaHCO_3$, KCl, $MgSO_4$, $CaCL_2$ and glucose, were purchased from Merck (Germany). Opioid receptor agonists (mu, delta, and kappa, respectively) [D-Ala_2, N-Me- Phe_4, Gly_5-O_l]-enkephalin (DAMGO), [D-pen]-enkephalin (DPDPE) and dynorphin-A; and antagonists including B-funaltrexamine hydrochloride (B-FNA), naltrindole hydrochloride (NTI), and Nor-binaltorphimine dihydrochloride (nor-BNI) were prepared from Bachem (Switzerland). Morphine sulfate was purchased from the Food and Drug Department of Urmia University of Medical Sciences (Urmia, Iran). Drugs were dissolved in LM-ACSF to designated concentrations (10 μM). During electrophysiological recordings, all the drugs were applied via the perfuse (10 μM), dissolved in LM-ACSF (Gáll et al., 2015).

2.1. Animals

The mice were maintained at 22 ± 2 °C under a 12-h light/dark cycle (light on at 0700 h) and given food and water ad libitum. All experimental protocols and procedures complied with the guidelines of the 1975 Declaration of Helsinki, as reflected in the guidelines of the Medical Ethics Committee, Ministry of Health, Iran, and were approved by the Research and Ethics Committee of Urmia University of Medical Sciences. All efforts were made to minimize the number of animals used. Studies were performed using male and female mice (n = 70) on postnatal day 14 (P14). Pups were kept in the animal house in a single cage with their mothers. On P14, the pups were randomly assigned to morphine-treated (n = 35) or a saline-treated control groups (n = 35). All injections were administered subcutaneously (s.c.) in a volume of 0.1 mL. The control group received daily injections of 0.9% physiological saline, and the morphine-treated group were injected with additive doses of morphine sulfate solution once a day for 5 consecutive days (2, 4, 8, 16, and 32 mg/kg) at 0800 h. Although most previous studies had used higher doses of morphine (> 32 mg/kg), we avoided such doses because they resulted in considerable toxicity symptoms and mortality in this study and our previous investigation (Saboory et al., 2012).

2.2. Slice preparation

Mice pups (n = 70), aged 19–20 days (P19–20), were anesthetized with ether and quickly decapitated, and the brain was rapidly removed and transferred into ice-cold 2–5 °C, continuously oxygenated with 95% O_2–5% CO_2 ACSF-composition (mM): 123 NaCl; 2.5 KCl; 1.5 $CaCl_2$; 2 $MgSO_4$; 25 $NaHCO_3$, 1.25 NaH_2PO_4, and 10 glucose (pH 7.35–7.45). The frontal lobe and the cerebellum were cut. Then, the obtained block was glued with cyanoacrylate to a Vibratome stage (Vibroslice, Campden instruments, UK) and submerged in ice-cold oxygenated ACSF and then sliced. The experiments were performed on horizontal hippocampal slices (400 μm) for LM-ACSF-induced seizure-like events (SLEs) and interictal bursts. The slices were incubated for at least 1.5 h at room temperature in oxygenated normal artificial cerebrospinal fluid (nACSF) (Gáll et al., 2015).

2.3. Electrophysiological recordings

Local field potential (LFP) recordings were performed with microelectrodes (3–8 MΩ) pulled from glass capillaries (TW150F-4; World Precision Instruments (WPI), Sarasota, FL, USA) using a one-stage pipette puller (Narishige, Japan), filled with nACSF, and positioned in the stratum pyramidale of hippocampal area CA_1 with a manual micromanipulator (WPI, Sarasota, FL, USA). Recordings were started after 10 min of accommodation in the interfaced-type chamber with the sampling rate of 1 kHz. An amplifier (ScienceBeam, Tehran, Iran) with a preamplifier was used (bandwidth 0.16 Hz–2 kHz, 2k gain) and a built-in 50 Hz notch filter. Data were digitized with the eTrace Analyzer software (ScienceBeam, Tehran, Iran) and stored on a computer hard disk for offline analysis. Recordings were performed from the CA1 pyramidal neuronal layer of the hippocampus. LM-ACSF was utilized to induce SLEs; and extracellular glass electrodes containing nACSF were positioned at locations yielding maximal field potential amplitudes in CA1 stratum pyramidale. All single-site recordings of SLEs were obtained from CA1 stratum pyramidale, filtered with 1 Hz low-pass and 1000 Hz high-pass filters unless stated otherwise, amplified, and recorded with an electromadule R12 and DAM80 amplifier (Axon Instruments, Foster City, CA).

2.4. Data analysis

Data distribution was checked using SPSS 22. The data that were not normally distributed were analyzed using nonparametric tests. The Wilcoxon test was employed for two dependent-group comparisons and Friedman test was used for multiple dependent comparisons. The results were expressed as mean ± SEM, and $P < 0.05$ was considered significant.

3. Results

In the majority of brain slices, perfusion with LM-ACSF elicited the status of recurrent discharges or status-like activity. The status of recurrent discharges consisted of short 1–10 s or less-than–1 s discharges with a negative discharges' shift repeated every 2–5 s and lasted as long as the [Mg^{2+}] in the ACSF remained low. In this study, the effects of selective agonist and antagonist of opioid receptors on SLEs in control and morphine-dependent mice varied greatly depending on their type. These results are summarized in Figs. 1–6 where it can be seen that mu, kappa, and delta opioid receptor agonists and antagonists were generally effective in evoking SLEs when perfused on hippocampal slices. In each of the 72 different brain slices, status epileptiform activity were consistent and did not change their electromorphologic

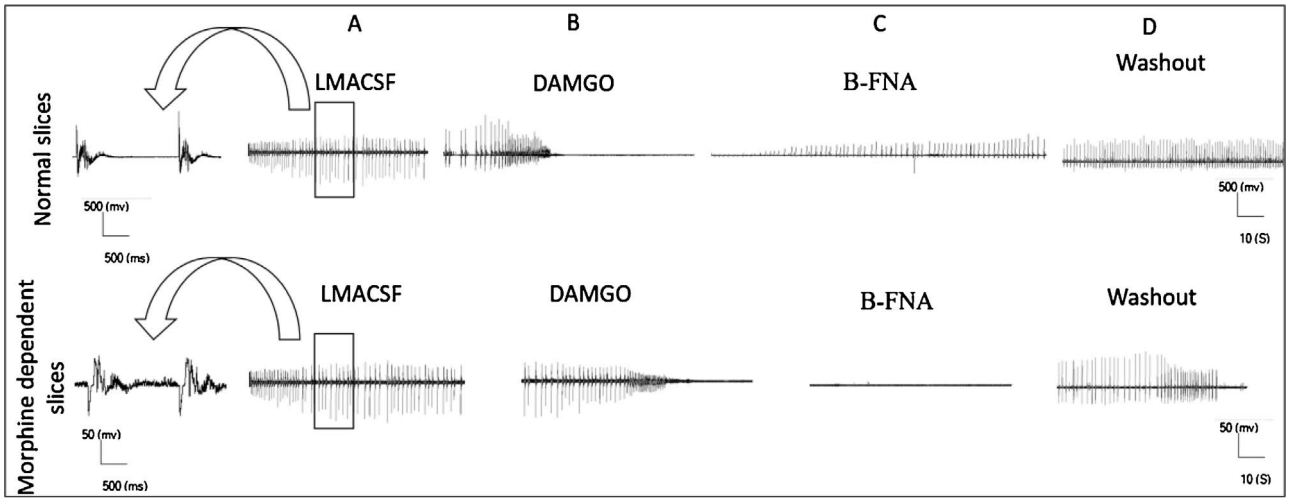

Fig. 1. Graphical illustration of selective MOR agonist (DAMGO) and antagonist (B-FNA) effects on LM-ACSF-induced SLEs in the brain slices of normal and morphine-dependent mice. A: SLEs recorded from CA1 area of hippocampus perfused with LM-ACSF; B: DAMGO 10 μM completely depressed LM-ACSF-induced SLEs; C: B-FNA 10 μM recovered the SLEs in normal slices but not in morphine-dependent ones; D: washout with LM-ACSF reversed the effects of drugs and re-induced the SLEs.

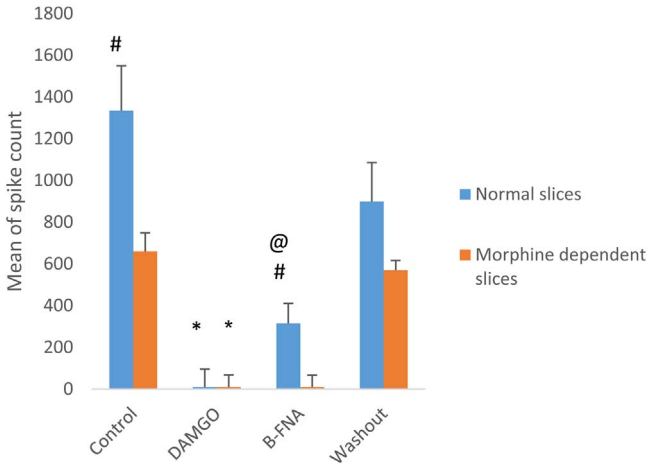

Fig. 2. Effects of MOR agonist (DAMGO) and antagonist (B-FNA) on SLEs in normal and morphine-dependent infant mice. In both groups, DAMGO suppressed SLEs and B-FNA recovered the activity in normal but not in morphine-dependent mice, whereas washout with LM-ACSF re-induced SLEs in both groups. The experiments were performed on n = 10 slices in control and n = 6 slices in other stages. * indicates significant difference from control in both series ($P < 0.01$); @ indicates significant difference from DAMGO in normal slices ($p = 0.035$); # indicates significant difference from morphine-dependent slices at the same stage ($P < 0.02$).

appearance. Status epileptiform activity in slices prepared from normal mice started approximately 5–10 min after the administration of LM-ACSF and lasted for approximately 95–120 min in a consistent and stable manner. However, in morphine-dependent slices, the activity started approximately 10–20 min after the administration of LM-ACSF and lasted as long as the [Mg^{2+}] in the ACSF remained low. Meanwhile, spike counts per time unit in normal brain slices were significantly higher than those of morphine-dependent slices.

Opioid receptor selective agonists and antagonists showed various effects in the concentration and duration used in this test. The MOR agonist (DAMGO) resulted in complete inhibition of SLEs created with LM-ACSF in slices from normal and morphine-dependent mice. Nevertheless, the selective antagonist of MOR (B-FNA) re-induced SLEs in normal mice, but suppressed this activity in morphine-dependent ones (Figs. 1 and 2).

Selective DOR agonist (DPDPE) decreased SLEs both in control and morphine-dependent brain slices. Selective DOR antagonist (NTI) decreased SLEs, but this suppression was not significant (Figs. 3 and 4).

The KOR agonist (dynorphin-A) and KOR antagonist (Nor-BNI) respectively increased and decreased SLEs both in the brain slices of normal and morphine-dependent mice (Figs. 5 and 6).

4. Discussion

In this study, the effects of selective agonists and antagonists of μ, κ and δ opioid receptors on LM-ACSF-induced SLEs were recorded in CA1 stratum pyramidale of hippocampal slices in normal and morphine-dependent infant mice. The present study confirms and further extends the existing findings by demonstrating that DAMGO and DPDPE selective agonists of MOR and DOR, respectively, are anti-seizure in mice, while Dyn-A selective agonist of KOR is convulsant. Furthermore, the selectivity of their anticonvulsant actions at KOR was demonstrated using nor-BNI selective KOR antagonist. Our results demonstrated that DAMGO and DPDPE would protect mice against convulsions, and that these effects could not be selectively blocked by their respective opioid antagonists such as B-FNA and NTI, respectively. This suggests that both MOR and DOR mediate the anticonvulsant properties of these ligands in this study, similar to flurothyl seizure models. These data are reinforced by other observations that MOR and DOR are involved in the opioid control of seizure protection. More recently, the results of experiments using the above-mentioned agonist and antagonist of opioid receptors showed that MOR and KOR have a pro-seizure effect and DOR has no effect (Saboory et al., 2007; Thompson et al., 2009); MOR and KOR have a pro-seizure effect and DOR has an anti-seizure effect (Feng et al., 2012); and MOR and DOR have a pro-seizure effect and KOR has an anti-seizure effect (Przewłocka et al., 1995; Yajima et al., 2000).

It has been reported that the chronic use of opioid receptor agonists such as morphine leads to remarkable down-regulation of corresponding receptors, which is one of the mechanisms of tolerance to opiates (Potschka et al., 2000). Meanwhile, it was reported that morphine-pretreated animals show a faster acquisition of seizure activity. The evaluation of the postictal seizure suppression after a fully kindled seizure demonstrates that morphine-pretreated rats have a decreased sensitivity to subsequent kindling stimulations (Hofmann et al., 2006). It has also been reported that early exposure to chronic morphine in infant rats might change their susceptibility to PTZ-induced seizure in an age-dependent manner (Gholami and Saboory, 2013; Gholami et al., 2014). Previous studies have demonstrated that chronic opiate exposure during infancy affects the developing brain and alters the number and sensitivity of opioid receptors throughout life (Thornton and Smith, 1998). Clinical studies have reported an increased prevalence of autonomic dysregulation, nystagmus, and strabismus in children ex-

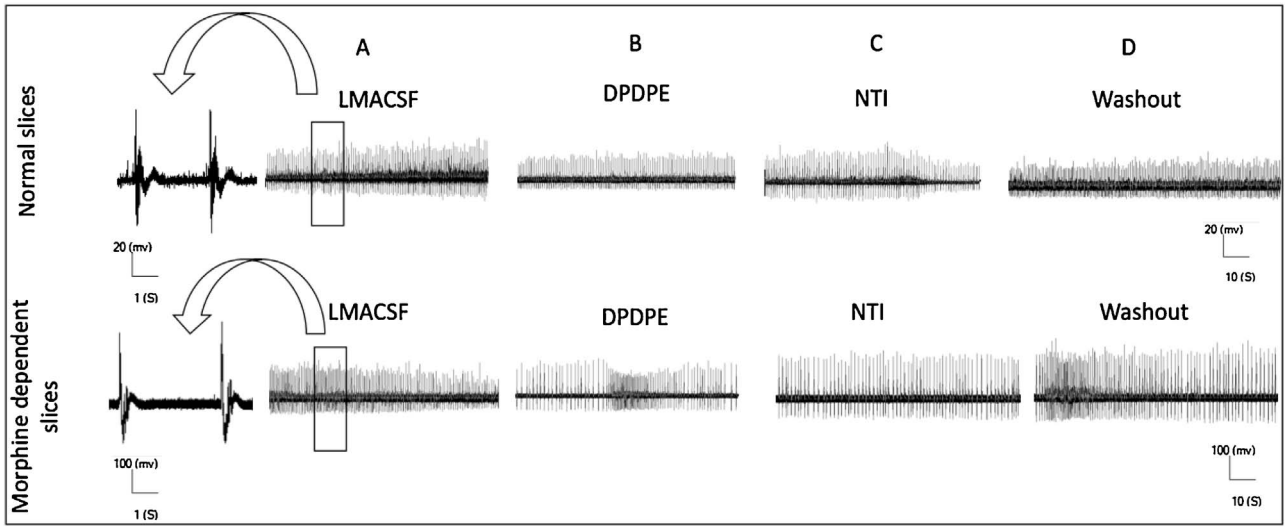

Fig. 3. Graphical illustration of DOR agonist (DPDPE) and antagonist (NTI) effects on LM-ACSF-induced SLEs in the brain slices of normal and morphine-dependent infant mice. A: SLEs recorded from CA1 area of hippocampus perfused with LM-ACSF; B: in both groups of slices, DPDPE attenuated LM-ACSF-induced SLEs; C: NTI did not show any remarkable changes on SLEs in either group; D: washout with LM-ACSF reversed the effects of drugs.

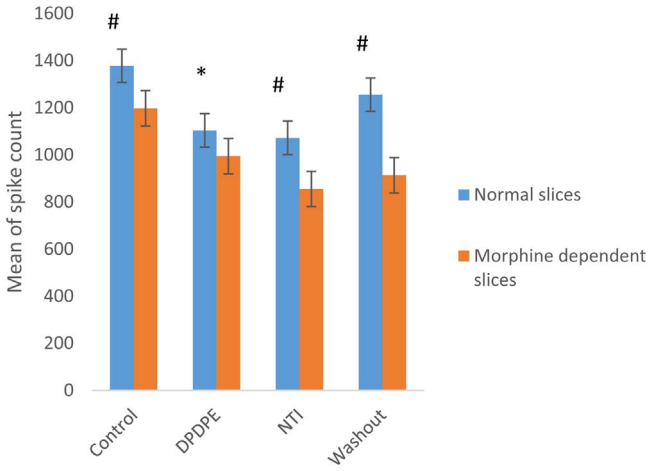

Fig. 4. Effects of DOR agonist (DPDPE) and antagonist (NTI) on SLEs in normal and morphine-dependent infant mice. In both groups, DPDPE decreased spike count number and NTI did not recover SLEs, but even potentiated the suppressive effect of DPDPE in morphine-dependent slices. However, washout with LM-ACSF recovered SLEs. The experiments were performed on n = 10 slices in control and n = 6 slices in other stages. * indicates significant difference from control in each group ($P < 0.02$); # indicates significant difference between the two groups at each stage ($P < 0.04$).

posed prenatally to opiates. At the pre- and elementary school ages, these children show motor and cognitive impairments, inattention, and hyperactivity (Ross et al., 2015). Repeated morphine exposure early in life could result in long-term alterations in the opioid response (Rozisky et al., 2008). Opioid receptor expression undergoes significant developmental regulation. Some studies have suggested that opioid receptor development occurs within the first 2 weeks of life (Nandi and Fitzgerald, 2005), and that MOR density and binding peaks at P7 and subsequently decreases to adult levels (Rahman et al., 1998). Chronic administration of morphine between postnatal days 1 and 8 produces a marked decrease in the number of brain MORs, which then gradually increases to control levels by day 14 of treatment. Longer treatment cannot produce further changes to opioid receptors. No significant changes to other receptors (δ-or κ) have been observed (Tempel and Espinoza, 1992). In a model of morphine sensitization in rats, autoradiography revealed a significant increase in the number of MORs in the hippocampus (Vigano et al., 2003) that making this system one of the predominant endogenous modulatory mechanisms that affect seizure susceptibility. Although the hippocampal formation is a structure that expresses significant densities of opioid receptors, morphine and other neurotransmitters including glutamate, acetylcholine, GABA, and nitric oxide appear to have a functional relationships (Saboory et al., 2014). The co-localization between hippocampal MOR and GABAergic interneurons is observed in CA1, CA3, and dentate gyrus regions in rats, suggesting that these receptors can directly control hippocampal GABAergic neuronal activity (Kalyuzhny and Wessendorf, 1998). Biochemical data indicate that acute morphine reduces synaptosomal calcium. Nevertheless, with the development of dependence, the calcium level in synaptosomes increases in a proportional way (Yang et al., 2010). Meanwhile, it has been reported that morphine, through the sustained activation of opioid receptors, can promote abnormal programmed cell death by enhancing the expression of pro-apoptotic Fas receptor protein and damping the expression of anti-apoptotic Bcl-2 oncoprotein. These changes might alter the seizure susceptibility of morphine treated mice (Boronat et al., 2001). Also, Chronic opiate exposure induces neuronal excitation, resulting in elevated intracellular Ca^{2+} levels, which are toxic to neurons. The pathological elevation of intracellular Ca^{2+} concentration is buffered by Ca^{2+} binding proteins such as Parvalbumin, Calretinin, Calbindin. Changes in the expression of Calcium binding proteins in specific regions of neonatal mouse brain have been reported (Maharajan et al., 2000; Mithbaokar et al., 2016).

Opiate depresses the stimulated release of the excitatory transmitter by decreasing the supply of Ca^{2+} ions to the stimulus-release coupling mechanism in sympathetic nerve terminals (Illes et al., 1980). In a study, tolerance was induced in rats by subcutaneous implantation of slow-release pellets of 75 mg of morphine over 8 days. No increased electrographic spiking occurred in these animals when compared to non-tolerant controls. However, when these animals were injected with morphine (300 mg/kg) and withdrawal was subsequently precipitated by naltrexone, there were significantly more seizures in the tolerant animals as compared to the non-tolerant ones (Frenk, 1983). While these studies do support the hypothesis that electrographic seizures elicited by i.c.v. endorphins and, to a lesser degree, i.c.v. morphine, are mediated by specific opiate receptors, it seems that these receptors are different from the MOR assumed to mediate the analgesic effects of opiates. This observation, together with the relatively high doses of naloxone needed to reverse the epileptogenic effects of enkephalins and morphine, suggested that DOR mediates these effects (Frenk, 1983). We found that pretreatment with systemic morphine delayed the onset and reduced the count of LM-ACSF-induced SLEs. It has been reported that

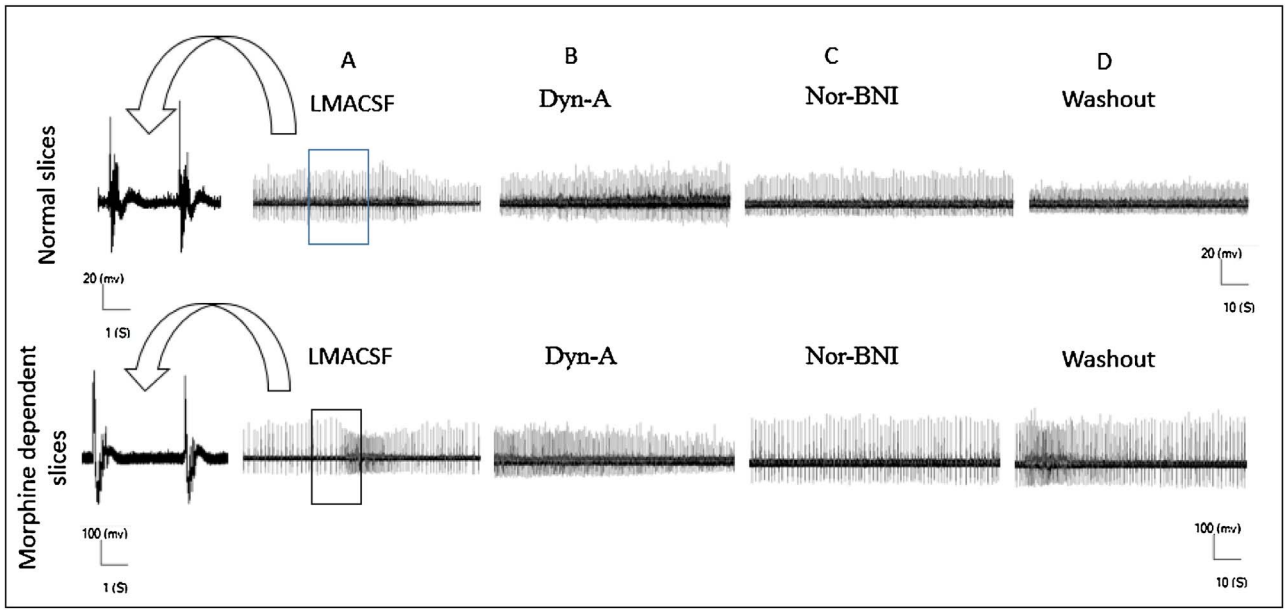

Fig. 5. Graphical illustration of selective KOR agonist (Dyn-A) and antagonist (Nor-BNI) effects on LM-ACSF-induced SLEs in the brain slices of normal and morphine-dependent infant mice. A: SLEs recorded from CA1 area of hippocampus perfused with LM-ACSF. B: Dyn-A potentiated the LM-ACSF-induced SLEs; C: Nor-BNI decreased the spike count; D: washout with LM-ACSF reversed the effects of drugs.

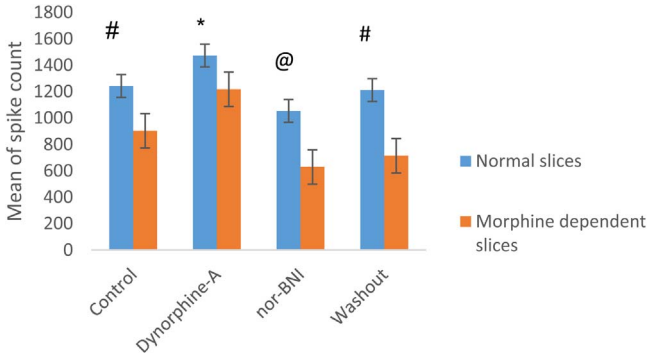

Fig. 6. Effects of selective KOR agonist (Dyn-A) and antagonist (nor-BNI) on SLEs in normal and morphine-dependent infant mice. In both groups, Dyn-A increased spike count number and nor-BNI decreased the effect of Dyn-A, whereas washout with LM-ACSF potentiated the SLEs. The experiments were performed on n = 10 slices in control and n = 6 slices in other stages. * indicates significant difference from control in both series; # indicates significant difference between normal and morphine-dependent slices at each identical stage ($P < 0.02$); @ indicates significant difference from Dyn-A in both series ($P < 0.04$).

morphine delays the onset of generalized myoclonus in the photosensitive baboon and naloxone reverses this effect (Hofmann et al., 2006). In a study similar to ours, the use of morphine during P8–P14 reduced seizure behavior and severity in adults, representing the long-term, anti-convulsant effect of morphine. Therefore, the result of the present study is consistent with findings of other investigations.

Both anti- and pro-convulsant effects of morphine in chemical and electrical seizure models can be reversed by MOR antagonists (Lauretti et al., 1994). This effect was not observed in the present study by B-FNA selective MOR antagonist in morphine-dependent brain slices but accrued in the normal group. It is possible that the mechanism of this action is mediated through inhibitory effects on glutamatergic excitatory pathways and increasing the GABAergic activity (Zieglgansberger et al., 1979). It has also been demonstrated that prenatal morphine exposure causes an up-regulation of α1 subunit and down-regulation of β2/γ2 subunit expression of GABA A receptors, associated with an increase in seizure susceptibility (Wang et al., 2011). In our study, it is therefore possible that following repeated early-life morphine exposure, alteration or desensitization of MOR might have led to the alteration of GABAergic transmission in the hippocampus, which may, in turn, have caused the anti-convulsant effects of mu and delta agonist and resulted in the ssuppression of GABAergic interneurons that decrease GABA release (Saboory et al., 2007). As was observed in this study, KOR may be involved in the excitatory effect of morphine on SLEs. Although the low Mg^{2+}/high K^{+} model-induced SLEs cannot fully reflect the mechanisms of epileptic syndromes, it is the most popular technique to obtain SLEs. The ultimate goal of this study was the analysis of SLEs by spike count which characterizes the pharmacologic action of opioid receptors' agonists and antagonist in normal and morphine-dependent infant mice. The pro- and anti-seizure actions of drugs were analyzed by evaluating the total suppression of SLEs. Based on the pattern of modifications, the pharmacological actions were sometimes pro-seizure rather than anti-seizure (Gáll et al., 2015). The presynaptic inhibitory effect of morphine is likely induced by the reduction of Ca^{2+} entrance into nerve terminals and, thereby, through the prevention of the release of glutamate in the cerebral cortex (Castillo et al., 2011). In addition, it is possible that the activation of NO system by different doses of morphine can lead to opposite effects on seizure susceptibility, and that NO might induce the anticonvulsant effect of morphine by an increase in GABAergic tone (Gholami et al., 2014; Homayoun et al., 2002). Moreover, a number of studies have shown an interaction between opioid and cholinergic systems (Walker et al., 1991) and reported the inhibitory effect of morphine on cholinergic activity in the hippocampus (Decker and McGaugh, 1991). The cholinergic activation of hippocampal circuits may recruit the opioid-mediated modulation of hippocampal network states. Results of the present study and others (Chen et al., 2007) demonstrate that opioid peptides with a pharmacological selectivity for MOR and DOR are anticonvulsant, and opioid peptides with a pharmacological selectivity for KOR are convulsant. It is interesting to note that MOR and DOR selectively hyperpolarize neurons throughout the CNS (Przewłocka et al., 1983) and that endogenous opioids are localized in fiber tracts throughout the brain and spinal cord, involved in the generation and spread of seizure activity (Klein et al., 2005). Therefore, it seems possible that opioid peptides may indeed function as endogenous anticonvulsants in the CNS, modulating the underlying mechanisms of seizure arrest and refractoriness which are critical to the suppression of convulsions. When we put all these results together, it appears that the administration of systemic morphine in the absence of additional convulsant

manipulations activates at least two epileptogenic mechanisms one of which is mediated by specific opiate receptors, while the other is not. These results seem to indicate that the anticonvulsant effects of opioids are mediated by specific opiate receptors which are probably MOR and DOR and their convulsant effects occur via KOR. We strongly encourage increased attention to the potential effects of prenatal opiate exposure on brain development. Current data are scarce and partly complicated by interpretational problems. This should motivate further investigation of the effects of prenatal opiate exposure in the brain, so that health-care providers and policy makers can be sufficiently informed when making treatment programs for pregnant opioid dependent women and their children (Thompson et al., 2009).

Conflict of interest statement

The authors have no conflicts of interest to declare regarding the study described in this article and the preparation of the article.

Ethical approval

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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