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Research Report

Effect of tooth pulp and periaqueductal central gray electrical stimulation on β-endorphin release into the fluid perfusing the cerebral ventricles in rats

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ABSTRACT

Our previous studies demonstrated that tooth pulp stimulation increases the pain threshold, whereas stimulation of the periaqueductal central gray (PAG) has the opposite effect. The aim of this study was to investigate the effect exerted by electrical stimulation of the nociceptive afferent terminals in the tooth pulp and analgesic electrical stimulation of the PAG on the release of immunoreactive β-endorphin (β-EP-IR) into the cerebrospinal fluid (CSF) and artificial cerebrospinal fluid (aCSF) perfusing the cerebral ventricles in rat, and to establish whether during such stimulation the μ-opioid receptor (MOR) was activated. The tongue jerk reflex was induced by dental pulp stimulation in rats under chloralose anesthesia. CSF was collected from the cerebello-medullary cistern, and then 30-minute perfusions of the lateral ventricles with aCSF were conducted with collection of perfusate portions from the cerebello-medullary cistern at rest (control), during electrical tooth pulp stimulation evoking the nociceptive tongue jerk reflex and during inhibition of that reflex with simultaneous stimulation of tooth pulp and the PAG. In the second series of experiments, a MOR-selective antagonist, β-funaltrexamine (β-FNA) was perfused through the cerebral ventricles 10 min before tooth pulp stimulation. β-EP-IR was determined in the collected CSF and aCSF perfusates by radioimmunoassay (RIA). Stimulation of tooth pulp with electrical impulses resulted in a significant increase of β-EP-IR. On the other hand, simultaneous tooth pulp and PAG stimulation inhibited β-EP-IR release into the fluid perfusing the cerebral ventricles. β-FNA blocked evoked tongue jerks (ETJ) induced by PAG stimulation, but no enhancing effect of β-FNA on β-EP-IR release into the perfusate was observed. The obtained results indicate that stimulation of the tooth pulp increases β-EP release significantly, whereas PAG stimulation significantly inhibits β-EP-IR release into the CSF, and that these effects are mediated by MOR. The results of the experiments allow to conclude that endogenous β-EP, released as a result of electrical tooth pulp stimulation in orofacial pain, diffuses through the cerebroventricular ependyma into the CSF and exerts a modulatory effect, mediated by MOR, alterating the properties of neurons in the trigeminal sensory nuclei, interneurons, and motoneurons of the hypoglossal nerve.

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1. Introduction

Endogenous opioid peptides, enkephalins, endorphins, endomorphins and dynorphins represent the group of bioregulatory factors with a wide range of biological activities (Toide, 2006). β-Endorphin (β-EP) acts as a neuromodulator in the central nervous system (CNS) (Bach, 1997; MacMillan et al., 1998; Höistad et al., 2005). Within the CNS of the rat, β-EP is mainly synthesized in the anterior lobe of the pituitary gland (Zakarian and Smyth, 1982), cell bodies clustered to the arcuate nucleus (ARC) of the mediobasal hypothalamus and in the ventral striatum (Roth-Deri et al., 2003). β-EP containing fibers spread widely in the brain but major fiber tracts projecting along the brain ventricles towards the periaqueductal central gray (PAG), are in the midbrain (Gioia and Bianchi, 1995; Khachaturian et al., 1993) which is of pivotal importance to the regulation of nociception (Yang et al., 2007b). Noxious stimuli may increase the release of endogenous opioid peptides, including β-EP, into the midbrain, PAG and hypothalamus (Yang et al., 2006), as well as into the CSF (Bach et al., 1995). β-EP exerts its analgesic effect by activation of μ- and δ-opioid receptors (MOR and DOR respectively), which belong to the family of G protein-coupled receptors and are distributed over the entire somatic–dendritic membrane of neurons in the PAG and in the rostral ventral medulla (RVM) (Jongeling et al., 2009; Terashvili et al., 2008; Tseng, 2001). β-EP-induced analgesia is abolished or attenuated by a general opioid antagonist, naloxone, by MOR-selective β-funaltrexamine (β-FNA) and by DOR-selective, naltrindole (NTI) (Pavlovic et al., 1996). The potent opioid action of β-EP and its anatomical distribution in neuron projections in the brain and brainstem, many of which terminate in the PAG (Reichling et al., 1988), suggest a probable role of β-EP in the regulation of afferent nociceptive processing.

The reflexes used for studying transmission of pain in brainstem are jaw-opening reflex (JOR) also known as trigemino-biventralis reflex (studies in cats) (Hu et al., 1986; Oliveras et al., 1974) and evoked tongue jerk reflex (ETJ) also known as trigemino-hypoglossal (studies in rats) (Leśnik and Traczyk, 1978; Zubrzycka et al., 1997). It was reported in the literature that both these reflexes were inhibited by the stimulation of the PAG and nucleus raphe magnus (NMR) (Dostrovsky et al., 1982; Hu et al., 1986; Zubrzycka and Janecka, 2000).

PAG stimulation through opioid-related mechanisms can suppress tongue and jaw reflexes, including those evoked by pulp stimulation, as well as suppress the responses of nociceptive neurons in the trigeminal sensory nuclei (Chiang et al., 1991; Chung et al., 1987; Dostrovsky et al., 1982; Hu et al., 1981; Oliveras et al., 1974; Sessle et al., 1981; Sheng et al., 2000; Sunakawa et al., 1999; Zhang et al., 1999).

In our previous studies, we have demonstrated that nociceptive stimulation of tooth pulp afferents enhanced the release of substance P (SP), whereas PAG stimulation inhibited its release to the aCSF (Zubrzycka and Janecka, 2002), but had no effect on the release of vasopressin (AVP) and oxytocin (OT) (Zubrzycka and Janecka, 2007). Here, we wanted to determine, in the same experimental model, to what extent noxious stimuli from the tooth pulp and electrical stimulation of the PAG can induce the release of β-EP into the artificial cerebrospinal fluid (aCSF) in rats.

2. Results

2.1. β-EP-immunoreactivity content in aCSF during electrical stimulation of tooth pulp and tooth pulp+PAG

β-EP immunoreactivity (β-EP-IR) was determined by radioimmunoassay. In the first series, CSF was collected from the cerebellomedullary cistern and then 30 min perfusions of the lateral ventricles with aCSF were conducted with collection of perfusate portions at rest (control), during electrical tooth pulp stimulation and during simultaneous tooth pulp and PAG stimulation. The experiments were performed on 7 animals (n=7). In Fig. 1 the average content of β-EP-IR in CSF and aCSF is shown before and after stimulation. Electrical stimulation of tooth pulp caused a significant increase of β-EP-IR content in aCSF, while the simultaneous stimulation of tooth pulp and the PAG had no effect on the level of β-EP-IR.

2.2. β-EP-immunoreactivity content in aCSF during simultaneous electrical stimulation of tooth pulp and PAG, following β-FNA perfusion of cerebral ventricles

In the second series, a MOR-selective antagonist, β-FNA (100 nmol/mL) was perfused through the cerebral ventricles for 10 min, preceding simultaneous tooth pulp and PAG stimulation. The average content of β-EP-IR in aCSF is shown in Fig. 2. The obtained results indicated that perfusion of cerebral ventricles with β-FNA had no effect on the release of β-EP during tooth pulp and PAG stimulation.

2.3. Effect of β-FNA on evoked tongue jerks during tooth pulp and PAG stimulation

This experiment was performed to determine whether β-FNA can abolish the influence of the PAG on nociceptive evoked tongue jerks (ETJ) induced by tooth pulp stimulation. The amplitude of evoked retractory movement of the tongue during electrical tooth pulp stimulation was recorded and

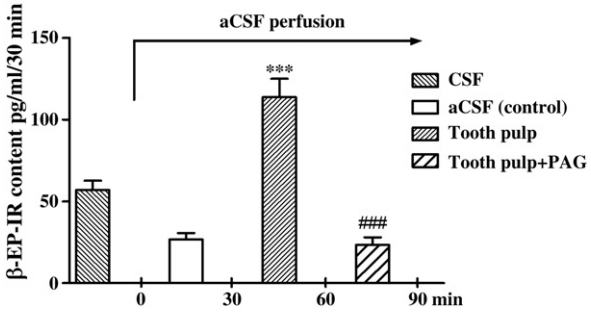

Fig. 1 – β-EP-IR content (pg/mL/30 min) in: CSF, aCSF perfusate without stimulation (0–30 min), aCSF perfusate during tooth pulp stimulation (30–60 min), and aCSF perfusate during tooth pulp and PAG stimulation (60–90 min). The data represent mean ± SEM of 7 rats per group. Statistical significance was assessed using one-way ANOVA and a post-hoc multiple comparison Student–Newman–Keuls test. ***, p<0.001 as compared with aCSF (control). ###, p<0.001 for tooth pulp and PAG vs. tooth pulp stimulation.