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Opioid peptides as possible neuromodulators in the frog peripheral nerve system

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Abstract

Sciatic nerves of the frog *Rana ridibunda* were examined for the effects of applied opioid peptide, methionine-enkephalin, synthetic enkephalin analogue, leucine-enkephalin-NH_2 and opiate antagonist, naloxone. The effect of both peptides in concentrations of 1×10^{-6} and 1×10^{-5} M or naloxone in 1×10^{-6} M was investigated on the action potential parameters using electrophysiological techniques.

The isolated nerves were stimulated by single square pulses each of which lasted for 0.5 ms at supramaximal strength. Effect of each single dose of peptides at 0 min was compared with the remaining time segments. Both peptides produced changes in action potential of nerve when compared with untreated nerves. Methionine-enkephalin in both concentrations reduced the amplitude between 7% and 41% and conduction velocity at about 26–61%. This peptide in the same concentrations prolonged the duration around 12–53% and increased the stimulating voltage at about 9–50%. In contrast, leucine-enkephalin-NH_2 in both concentrations caused a decrease in amplitude between 13% and 48% and in conduction velocity around 20–50%. The same concentrations of this peptide prolonged the duration at about 3–33% and increased the stimulating voltage at about 10–56%, but naloxone in 1×10^{-6} M antagonized the responses of both peptides over 75%.

The results indicate that both opioid peptides produce changes in action potential parameters in frog peripheral nerve system and these changes are partially reversed by naloxone.

Keywords: Electrophysiology; Neuromodulators; Methionine-enkephalin; Leucine-enkephalin-NH_2, *Rana ridibunda*; Sciatic nerve

1. Introduction

Endogenous opioids regarded as a family of peptides are widely distributed throughout the central and peripheral nervous system and seem to play a multifunctional role in the regulation of these neuronal processes. These biologically active peptides were isolated from vertebrates and can mainly be grouped into enkephalins, endorphins and dynorphins. In mammals opioid peptides originate from different precursors and interact with at least three different receptor subtypes; mü (μ), delta (δ) and kappa (κ) (for reviews; Evans et al., 1988; Chiou, 2000; Bodnar and Klein, 2005).

The opioid peptides Leu-Enk (leucine-enkephalin) and Met-Enk (methionine-enkephalin) identified at first in the brain of mammals belong to a new neuromodulator family (Hughes et al., 1975). Based on immunohistological procedures opioid peptides have been demonstrated in the nervous systems of the guinea pig, hamster and mouse (Watson et al., 1977; Van Leeuwen et al., 1983; Gaymann and Martin, 1987; D'Este et al., 2002; Racz and Halasy, 2003; Kovacs et al., 2005). Fur-

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thermore, numerous opioid peptide containing neurons have been observed by immunohistochemical techniques in brain, spinal cord and peripheral nervous system of rat (Simantov et al., 1977; Rossier et al., 1977; Hughes et al., 1977). Also, opioid peptides were identified in oxytocine and vasopressine terminals of the rat neurohypophysis (Martin et al., 1983).

Opioid peptides identified in the mammals are also present in amphibians. They have been demonstrated in the brain of *Rana catesbeiana* and *R. esculanta* (Kanetoh et al., 2003; Sabbieti et al., 2003), in the gastrointestinal tract of *R. temporaria* (Valverde et al., 1993) and in the adrenal organs of *R. esculanta, Caldula pulchra* and *Bufo marinus* (Reinecke et al., 1992). In addition, the presence of opioid peptide immuoreactivity in *R. ridibunda* (Leboulenger et al., 1983) and *R. catesbeiana* (Kondo and Yui, 1984; Kuramoto, 1987) has been reported. Opioids in *R. nigromaculata* have been characterized in the hypothalamus and the hypophysis (Takeda et al., 1990). Further opioids have been identified in the retina, skin and nerve tissues of the various *Phyllomedusa* species (Jackson et al., 1980; Cone and Goldstein, 1982; Stevens and Yaksh, 1986; Erspamer, 1994).

Opioids have a variety of physiological role in vertebrates, but their effects differ depending on the experimental animals and their tissues. They are known to act differently on sensitive smooth muscle preparation depending on animals; while opioids stimulate the gut contraction in guinea pig, dogs and rats (Paton, 1957; Cox, 1988), they inhibit the muscle contraction in mouse vas deferens, but the inhibiting effect was found to be reversed by naloxone (Hughes et al., 1975; Henderson, 1976). Furthermore, opioids inhibit the action potential in squid giant axons (Fraizer et al., 1972), in guinea pig, cat sural nerve (Soteropoulus and Standaert, 1973), in rabbit vagus nerve (Jurna and Grossman, 1977) and additionally in peripheral frog nerves (Çamlica et al., 2004). However, the previous investigations suggest that there is no information on their physiological relevance on the duration and stimulating voltage of action potential in *R. ridibunda* nerve. The present study was, therefore undertaken to investigate the influence of Met-Enk, Leu-Enk-NH_2 or their interactions with opiate antagonist, naloxone, especially on these parameters, but also on the amplitude and conduction velocity of action potential in peripheral nerve system.

2. Materials and methods

2.1. Tissue preparation

The adult frogs *R. ridibunda* were maintained at 20–24 °C on a 12 h photoperiod in a tank with river water for two weeks prior to experimentation. For this experiment 52 animals weighing 35–40 g were used (care and experimentation of the animals were in accordance with the principles of laboratory animal care, NIH). After decapitation the isolated sciatic nerves were removed (Kleinelb, 1991) and placed in petri dishes containing Ringer's solution (NaCl, 118.87 mM; KCl, 2.47 mM; $CaCl_2$, 1.08 mM; $NaHCO_3$, 2.38 mM; pH 7.2) at 4–6 °C for a day before start of experiment. All experiments were carried out at room temperature, 20–24 °C.

2.2. Drugs and treatment

The drugs used in this study were Met-Enk, Leu-Enk-NH_2 and naloxone obtained from Sigma 2005 (Chemical Co.; Met-Enk, M 6638; Leu-Enk-NH_2, E 3756; naloxone, N 7758). The isolated nerves were divided into eight groups. Two of them were for controls (Groups 1 and 2: without the drug treatment), two were for Met-Enk (Group 3: 1×10^{-6} M; Group 4: 1×10^{-5} M), one was for Met-Enk plus naloxone (Group 5: 1×10^{-6} M plus 1×10^{-6} M) as well as two of them were for Leu-Enk-NH_2 (Group 6: 1×10^{-6} M; Group 7: 1×10^{-5} M) and the last one was for Leu-Enk-NH_2 plus naloxone (Group 8: 1×10^{-6} M plus 1×10^{-6} M). The treatment of nerves with drugs was undertaken in petri dishes.

2.3. Stimulating and recording

The experiments were carried out at the laboratory of biophysics in the Medical Faculty of Mersin University using extracellular recording techniques (Katz, 1966; Andrew, 1972). For in vitro experiments a 5×15 cm plexiglass nerve chamber was used containing Ag/AgCl electrodes. The distance between the electrodes was 0.5 cm. The stimulating voltage was set to produce a maximal compound action potential using single square pulses of supramaximal strength and 0.5 ms in duration. The nerve action potentials were recorded using a BIOPAC MP 100 Acquisition System Version 3.5.7 (Santa Barbara, USA).

After 120 min of stabilization in petri dishes the single nerve specimens were placed in nerve chamber and the compound action potentials (CAP) were recorded from each nerve before addition of drugs and these data were accepted as control. After recordings of CAP in control condition the nerves were exposed as follows to test solutions containing Met-Enk or Leu-Enk-NH_2 as well as Met-Enk or Leu-Enk-NH_2 plus naloxone (1×10^{-6} M, 1×10^{-5} M and 1×10^{-6} M plus 1×10^{-6} M) for different durations 30, 60, 90 and 120 min, respectively. The nerves were then stimulated and the action potential was recorded in all groups. BIOPAC Acqknowledge Analysis Software (ACK 100 W) was used to measure CAP parameters, such as the amplitude, conduction velocity, duration and the stimulating voltage. Conduction velocity was measured from the latencies of action potentials recorded with supramaxi-