Please cite this article in press as: Palus K et al. Enkephalin and neuropeptide-Y interaction in the intergeniculate leaflet network, a part of the mammalian biological clock. Neuroscience (2016), http://dx.doi.org/10.1016/j.neuroscience.2016.11.034

Neuroscience xxx (2016) xxx–xxx

ENKEPHALIN AND NEUROPEPTIDE-Y INTERACTION IN THE INTERGENICULATE LEAFLET NETWORK, A PART OF THE MAMMALIAN BIOLOGICAL CLOCK

K. PALUS,[a] L. CHROBOK,[a] M. KEPCZYNSKI[b] AND M. H. LEWANDOWSKI[a]*

[a] *Department of Neurophysiology and Chronobiology, Jagiellonian University in Krakow, Gronostajowa 9, 30-387 Krakow, Poland*

[b] *Faculty of Chemistry, Jagiellonian University in Krakow, Ingardena 3, 30-060 Krakow, Poland*

Abstract—The intergeniculate leaflet (IGL) is a flat thalamic nucleus implicated in the modulation of circadian rhythmicity. In rat, two main GABAergic subpopulations can be distinguished in the IGL: neurons synthesizing neuropeptide Y (NPY), which directly innervates the suprachiasmatic nuclei, and enkephalinergic cells, which connect contralaterally located leaflets. The aim of this study was to evaluate possible effects of inner IGL neurotransmitters on the spontaneous and synaptic activity of IGL neurons. The data presented in this article provide evidence that enkephalin, and not NPY, could act upon the majority of IGL neurons. Moreover, we investigated the type of opioid receptor activated by enkephalin and showed that the μ-receptor is functionally predominant in the IGL. The application of met-enkephalin not only robustly hyperpolarized IGL neurons (both putatively NPY-synthesizing and putatively enkephalinergic neurons), but it also was able to inhibit GABAergic and glutamatergic synaptic transmission. Based on this and previous studies, we hypothesize that IGL enkephalinergic neurons may act as powerful interneurons that inhibit themselves and NPY-synthesizing neurons, also in the contralaterally located IGL.

Key words: intergeniculate leaflet, enkephalin, neuropeptide Y, synaptic transmission, patch clamp.

*Corresponding author.
E-mail address: marian.lewandowski@uj.edu.pl (M. H. Lewandowski).

Abbreviations: ACSF, artificial cerebro-spinal fluid; AP-5, 2-amino-5-phosphonopentanoic acid; Bic, bicuculline methiodide; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; EGTA, ethylene glycol tetraacetic acid; ENK, enkephalin; GABA, γ-aminobutyric acid; GGT, geniculo-geniculate tract; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; I_A, A-type potassium current; IGL, intergeniculate leaflet; ISO, infra-slow oscillations; I_T, T-type calcium current; ME, met-enkephalin; mEPSC, miniature excitatory postsynaptic current; mIPSC, miniature inhibitory postsynaptic current; nACSF, normal artificial cerebro-spinal fluid; NAL, naltrindole hydrochloride; NAX, naloxonazine dihydrochloride; NPY, neuropeptide Y; SCN, suprachiasmatic nucleus; SD, standard deviation; SEM, standard error of the mean; TTX, tetrodotoxin citrate; ZT, Zeitgeber Time; βFNA, β-funaltrexamine hydrochloride.

http://dx.doi.org/10.1016/j.neuroscience.2016.11.034

INTRODUCTION

The intergeniculate leaflet (IGL) is a brain structure located in the lateral geniculate complex of the thalamus (Moore and Card, 1994). As the homologous pregeniculate nucleus in primates, the rodent IGL comprises two main neuronal subpopulations, both co-synthesizing γ-aminobutyric acid (GABA) (Moore, 1989; Moore and Speh, 1993; Lima et al., 2012). The majority of IGL neurons express enkephalin, and this subpopulation in rat forms the geniculo-geniculate tract (GGT; Mantyh and Kemp, 1983; Mikkelsen, 1992; Harrington, 1997), whereas in hamster, enkephalinergic fibers were additionally found to reach multiple brain nuclei (Morin and Blanchard, 1995, 1997, 2001). The second IGL neuronal subpopulation can be differentiated by the neuropeptide Y (NPY) presence (Moore and Card, 1994; Harrington, 1997). NPY-positive neurons form the geniculo-hypothalamic tract, sending information to the master generator of circadian rhythms, the suprachiasmatic nucleus (SCN) (Card and Moore, 1989; Glass et al., 2010). Therefore, the postulated function of the whole IGL is the modulation of circadian rhythmicity (Harrington and Rusak, 1986; Morin and Allen, 2006).

Enkephalin is an opioid peptide that binds to three classes of opioid receptors (δ, μ and κ), with the highest affinity to δ-receptors and the lowest to κ-receptors (Martin et al., 1976; Kosterlitz, 1985). Opioid receptors can localize both on the postsynaptic site and presynaptic terminals (Cohen et al., 1992; Stanford and Cooper, 1999). In the rat lateral geniculate complex the expression of δ-, μ- and κ-receptors have been reported (Mansour et al., 1994), whereas in hamster the δ-receptor has been found in the IGL (Byku et al., 2000). In the hamster SCN the δ-receptor has a predominant role in the actions of enkephalin such as non-photic phase shifting and the inhibition of light-induced phase shifting (Byku and Gannon, 2000a,b; Tierno et al., 2002). It has been hypothesized that opioid receptors in SCN could be localized on the presynaptic terminals of retino-hypothalamic tract (Tierno et al., 2002). NPY released in SCN from IGL-derived neuronal fibers binds mainly to Y_1 and Y_5 receptors, modulating the phase shifts to light, causing non-photic phase shift (Lall and Biello, 2003; Fetissov et al., 2004).

Studies performed in our laboratory have shown that enkephalinergic IGL neurons are characterized by the infra-slow oscillations (ISO) on the level of neuronal firing in the constant illumination conditions. ISO can be described as the high-frequency burst regularly followed by the silent period in the range of about 120 s (< 0.01 Hz) (Lewandowski et al., 2000, 2002; Lewandowski and Blasiak, 2004). This activity pattern is present only at *in vivo* not in *in vitro* preparation (Blasiak and Lewandowski, 2004a; Szkudlarek and Raastad, 2007). It has been previously hypothesized that enkephalin, together with GABA, could be responsible for the silent phase of this activity pattern (Lewandowski et al., 2002; Blasiak and Lewandowski, 2013). It has been settled that this IGL subpopulation is retinorecipient and activated by light, unlike neurons synthesizing NPY which are suppressed, nonresponsive or show complex reactions to light (Zhang and Rusak, 1989; Takatsuji et al., 1991; Thankachan and Rusak, 2005; Juhl et al., 2007). Lately, we have shown that NPY-positive neurons can be distinguished by the expression of A-type potassium current (I_A), while the majority of IGL neurons (possibly enkephalinergic subpopulation) can be characterized by T-type calcium current (I_T) (Chrobok et al., 2016).

The well-established function of the IGL is to collect and integrate photic and non-photic cues in order to send the consolidated information to the SCN (Harrington, 1997; Morin and Blanchard, 1998, 2005). The previous papers from our group show that IGL neurons are sensitive to neurotransmitters as: acetylcholine (Pekala et al., 2007; Werhun and Lewandowski, 2009), serotonin (Blasiak et al., 2006; Palus et al., 2013), orexins (Pekala et al., 2011; Palus et al., 2015b) or relaxin-3 (Blasiak et al., 2013), conveying non-photic information for the IGL network. Although, the only inner neurotransmitter with the documented electrophysiological action in the IGL is GABA (Blasiak and Lewandowski, 2004b; Palus et al., 2015b) and the electrophysiological actions of enkephalin and NPY within the neuronal network of IGL remain poorly understood. Therefore, the neuronal mechanism leading to the integration of upcoming information to the IGL is still not clear.

The aim of this study was to evaluate the possible electrophysiological effects of two inner neurotransmitters: enkephalin and NPY on the activity of a single, immunohistochemically identified IGL neuron. Moreover, our research was scoped on the identification of the receptor involved in the major inner neurotransmission. Data presented in this article may contribute to the explanation on how the information is being processed in the neuronal network of the IGL.

EXPERIMENTAL PROCEDURE

Brain tissue preparation

All experiments were performed in accordance with the European Community Council Directive of 24 November 1986 (86/0609/EEC) and Polish Animal Welfare Act of 23 May 2012 (82/2012). They were approved by the Local (Krakow) Ethical Commission. Every effort was made to minimize the number of animals used and their suffering. Animals were maintained on a 12-h light/dark cycle (lights on at 8.00 am, lights off at 8.00 pm) with *ad libitum* access to water and food in the Jagiellonian University animal facility.

In order to obtained brain slices containing IGL we used procedure described previously in detail (Palus et al., 2015b). In short, male (76 animals) Wistar rats (13–18 days old) were anesthetized with isoflurane (2 ml/kg body weight, Baxter) and decapitated between 1 and 2 h Zeitgeber Time (ZT 1–2, 9:00–10:00 am, light on 8:00). The brain was quickly removed from the skull and immersed in ice-cold, oxygenated (carbogen, 95% O_2, 5% CO_2) normal artificial cerebrospinal fluid (nACSF), composed of (in mM): 118 NaCl, 25 $NaHCO_3$, 3 KCl, 1.2 NaH_2PO_4, 2 $CaCl_2$, 2 $MgCl_2$ and 10 glucose. Then, the block of brain tissue containing the thalamus was placed on the cold plate of a vibroslicer (Leica VT1000S, Heidelberg, Germany). Coronal slices, 250 μm in thickness, including the IGL were cut and transferred to the pre-incubation chamber for 30 min at 32 °C and another 60 min at room temperature, then placed in the recording chamber (21–24 °C).

Whole-cell recordings

Recording electrodes were placed in the IGL under visual microscopic control. Single IGL neurons were identified under 40× objective of a Zeiss Examiner microscope fitted with infrared differential interference contrast (Göttingen, Germany). Recordings were performed in whole-cell configuration obtained by applying negative pressure from an Ez-gSEAL100B Pressure Controller (Neo Biosystem, San Jose, USA). The recorded signal was amplified by a SC 05LX (NPI, Tamm, Germany) amplifier, low-pass filtered at 3 kHz and digitized at 20 kHz. Spike2 and Signal (CED, Cambridge, UK) software were used for the recordings. Additionally, at the beginning of each recording voltage clamp depolarizing steps (15 steps, 200 ms duration, 700 ms interval) were given at 3 mV increments (first step −80 mV) from the holding potential −115 mV, in order to assign recorded IGL neurons into two groups (Chrobok et al., 2016). A liquid junction potential of approximately −15 mV was added to the measured membrane potential. In case of active neurons recorded in nACSF, frequency of action potential was measured (expressed in Hz). The membrane potential (expressed in mV) was examined from silent neurons in the nACSF and all neurons recorded in the presence of the tetrodotoxin (TTX, 0.5 μM). Typical recordings lasted ∼1000 s (for single drug application) or ∼2500 s (for double drug application).

Current clamp recordings. Experiments in the current clamp mode (holding current = 0 pA) were conducted at room temperature (21–24 °C). 70 male Wistar rats were sacrificed for all current clamp protocols. Patch pipettes were filled with normal intrapipette solution containing (in mM): potassium gluconate 125, KCl 20, HEPES 10, $MgCl_2$ 2, Na_2ATP 4, Na_3GTP 0.4, EGTA 1 and 0.05% biocytin (pH = 7.4 adjusted with 5 M KOH; osmolality ∼300 mOsmol/kg). Rectangular current pulses (1 s,

Please cite this article in press as: Palus K et al. Enkephalin and neuropeptide-Y interaction in the intergeniculate leaflet network, a part of the mammalian biological clock. Neuroscience (2016), http://dx.doi.org/10.1016/j.neuroscience.2016.11.034