Neuropeptides xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Neuropeptides

journal homepage: www.elsevier.com/locate/npep

The effects of endothelin-1 on satellite glial cells in peripheral ganglia

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ARTICLE INFO

Article history:
Received 17 November 2016
Received in revised form 1 March 2017
Accepted 16 March 2017
Available online xxxx

Keywords:
Endothelin
Satellite glial cells
Trigeminal ganglion
Nodose ganglion
Superior cervical ganglion
Sympathetic ganglia
Calcium imaging
Gap junctions

ABSTRACT

Endothelins (ET) are a family of highly active neuropeptides with manifold influences via ET receptors (ETR) in both the peripheral and central nervous systems. We have shown previously that satellite glial cells (SGCs) in mouse trigeminal ganglia (TG) are extremely sensitive to ET-1 in evoking $[Ca^{2+}]_{in}$ increase, apparently via ET_BR activation, but there is no functional information on ETR in SGCs of other peripheral ganglia. Here we tested the effects of ET-1 on SGCs in nodose ganglia (NG), which is sensory, and superior cervical ganglia (Sup-CG), which is part of the sympathetic nervous system, and further investigated the influence of ET-1 on SGCs in TG. Using calcium imaging we found that SGCs in intact, freshly isolated NG and Sup-CG are highly sensitive to ET-1, with threshold concentration at 0.1 nM. Our results showed that $[Ca^{2+}]_{in}$ elevation in response to ET-1 was partially due to Ca^{2+} influx from the extracellular space and partially to Ca^{2+} release from intracellular stores. Using receptor selective ETR agonists and antagonists, we found that the responses were mediated by mixed ET_AR/ET_BR in SGCs of NG and predominantly by ET_BR in SGCs of Sup-CG. By employing intracellular dye injection we examined coupling among SGCs around different neurons in the presence of 5 nM ET-1 and observed coupling inhibition in all the three ganglion types. In summary, our work showed that SGCs in mouse sensory and sympathetic ganglia are highly sensitive to ET-1 and that this peptide markedly reduces SGCs coupling. We conclude that ET-1, which may participate in neuron–glia communications, has similar functions in wide range of peripheral ganglia.

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1. Introduction

The endothelins (ETs) are a family that comprises three isoforms of 21-amino acid cyclic peptides (ET-1–3), which have a variety of functions mediated by two receptor subtypes, ET_A and ET_B (Rubanyi and Polokoff, 1994; Davenport et al., 2016). ETs act on the endothelium in blood vessels and on numerous other cell types including smooth muscles, astrocytes and neurons, and exert a variety of actions on the central and the peripheral nervous systems (Katzung, 2004; Jandeleit-Dahm and Watson, 2012; Smith et al., 2014; Davenport et al., 2016). ET-1, which activates both receptor types, is found throughout the pain pathways and has been the most studied isoform (Barr et al., 2011; Smith et al., 2014). In animal models, exogenous ET-1 inhibits pain when injected centrally (Yamamoto et al., 1994; D'Amico et al., 1997) or induces pain when injected peripherally (Piovezan et al., 2000). Also, it has been reported that peripheral administration of ET receptor (ETR) antagonists can alleviate acute and chronic pain (Khodorova et al., 2009a). ET-1 has been shown to directly activate nociceptors in humans and animals (Gokin et al., 2001; Namer et al., 2008). In addition, ET-1 potentiates nociception induced by other algogens such as formalin, serotonin, and capsaicin (Piovezan et al., 1997).

It appears that the ET_AR activation is associated with nociception, whereas the role of the ET_BR is less clear and varies between organs, the type of pain, and across species (Smith et al., 2014). On one hand it has been shown that ET_BR activation inhibits nociception by inducing the release of endorphins from keratinocytes (Khodorova et al., 2009b) and that a selective ET_BR antagonist enhances nociception (Imhof et al., 2011), but on the other hand there is strong evidence for the contribution of ET_BR to pain (e.g., Chichorro et al., 2009, 2010) and for the involvement of these receptors in inflammatory processes and in mediation of inflammatory pain (Griswold et al., 1999). Taken together these studies suggest that ET_BR have mixed actions (Khodorova et al., 2009b; Davenport et al., 2016).

Most of the studies on the role of ETR in pain signaling have focused on neurons. However, in the dorsal root ganglion (DRG), in addition to ET_AR immunoreactivity found in primary sensory neurons, ET_BR are present in satellite glial cells (SGCs) and nonmyelinating Schwann cells (Pomonis et al., 2001). Also, intracellular calcium ($[Ca^{2+}]_{in}$) elevation was observed in response to ET-1 in SGCs of DRG cultures (Vellani et al., 2011). In contrast to DRG, in the trigeminal ganglia (TG) not only SGCs, but also a subclass of neurons expressed ET_BR (Chichorro et al., 2009, 2010). In our previous work (Feldman-Goriachnik and Hanani, 2011) we showed that SGCs in

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http://dx.doi.org/10.1016/j.npep.2017.03.002
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Please cite this article as: Feldman-Goriachnik, R., Hanani, M., The effects of endothelin-1 on satellite glial cells in peripheral ganglia, Neuropeptides (2016), http://dx.doi.org/10.1016/j.npep.2017.03.002

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mouse TG are extremely sensitive to ET-1 in evoking $[Ca^{2+}]_{in}$ increase, apparently via ET_BR activation. Yet, the functional implications of this finding remain to be explored and there is no information on the presence of ETR in SGCs of other peripheral ganglia.

It is now recognized that ET-1 is produced by a variety of cells and there is evidence that neurons in DRG (Giaid et al., 1989) and TG (Milner et al., 2000a) contain ET-1. Therefore, it is very likely that ET-1 can function as a messenger between neurons and SGCs, which is in accordance with the current view that SGCs have a role in pain signaling (Ohara et al., 2009; Huang et al., 2010).

The aim of this study was to test the effect of ET-1 on SGCs in other peripheral ganglia: nodose ganglia (NG), which is sensory, and superior cervical ganglia (Sup-CG), which is part of the sympathetic nervous system and to further investigate the influence of ET-1 on SGCs in TG. Our hypothesis is that properties of SGCs in the sympathetic ganglion will be different from those in the sensory ganglia.

2. Materials and methods

2.1. Animals

The procedures were approved by the Animal Care and Use Committee of the Hebrew University, Hadassah Medical School and conform to the National Institutes of Health standards for the care and use of laboratory animals. Balb/c mice 3–5 months old of either sex (males:females about 1:1), weighing 19–23 g, were used ($N = 49$). Preliminary experiments showed that the results for females and males were not different. The animals were euthanized with CO_2 and the TG, NG or Sup-CG were removed and fixed to the bottom of a silicon rubber-coated dish using fine pins.

2.2. Ca^{2+} imaging

For Ca^{2+} microfluorimetry SGCs in intact ganglia were loaded with the Ca^{2+} indicator Fluo-3 AM [10 μM; Invitrogen (www.invitrogen.com)] in minimum essential medium-alpha for 70 min in an incubator at 37 °C. The dye is preferentially taken up by SGCs, which allows the recording of Ca^{2+} signals from these cells without interference from the neurons (Weick et al., 2003). Ganglia were mounted in a recording chamber on the stage of an Axioskop FS microscope (Zeiss, Jena, Germany) and superfused at 4 mL/min with Krebs solution, which contained (mM): NaCl (118), KCl (4.7), $NaHCO_3$ (14.4), $MgSO_4$ (1.2), NaH_2PO_4 (1.2), $CaCl_2$ (2.5), and glucose (11.5); pH 7.3, saturated with 95% O_2/5% CO_2. ET-1 (Sigma-Aldrich, St. Louis, MO, USA) or ET_BR agonist IRL-1620 (Tocris, www.tocris.com) was applied by rapidly changing the bath solution. Antagonists for ET-1: the ET_BR antagonist IRL-2500 or the ET_AR antagonist BQ-123 (Tocris, www.tocris.com) were added into the bathing medium 15 min before testing their effects. Doses and timing were chosen according to our previous work (Feldman-Goriachnik and Hanani, 2011) and preliminary tests. Images were acquired with cooled CCD camera (PCO, Kelheim, Germany), using Imaging Workbench 5 software (www.imagingworkbench.com). Fluorescence was excited at 450–490 nm, and emitted fluorescence (above 520 nm) was increased by elevated $[Ca^{2+}]_{in}$. Images were recorded at 0.3 Hz. The fluorescence ratio F/F_0, where F_0 is the baseline, was used to describe relative changes in $[Ca^{2+}]_{in}$. As the baseline level is 1, the response magnitude was defined as F/F_0-1. About 40 SGCs were imaged simultaneously in each field. For the experiments with low extracellular Ca^{2+} concentration we used 0.5 mM Ca^{2+}, and added 6 mM sucrose to the medium to balance the osmolarity.

2.3. Intracellular labeling

The dish with the ganglia was placed on the stage of an upright microscope, equipped with fluorescent illumination and a digital camera connected to a personal computer. The dish was superfused with Krebs solution, as described above. Individual SGCs were injected with the fluorescent dye Lucifer yellow (LY), 3% in 0.5 M LiCl solution from sharp glass microelectrodes, connected to a preamplifier (NeuroData Instrument Corp., New York, NY, USA). The dye was passed by hyperpolarizing current pulses, 100 ms in duration; 0.5 nA in amplitude at 10 Hz for 3–5 min. Dye injections were made under visual inspection to allow cell identification. After the injection of each cell, we checked whether labeled SGCs were present around neighboring neurons as a result of dye passage from the injected cell. Dye coupling data were pooled from multiple experiments. About 40–90 SGCs were injected per experimental group.

2.4. Statistical analysis

Unpaired Two-tailed *t*-Test or One-way ANOVA with Dunnett's Multiple Comparison Test was used to analyze the data; P value of <0.05 was considered as statistically significant. For the dye coupling experiments when an LY-injected cell was found to be dye-coupled, it was marked as 100, and when it was not coupled, as 0. Values are expressed as mean ± SEM.

3. Results

3.1. Ca^{2+} responses of SGCs to ET-1

Our previous study (Feldman-Goriachnik and Hanani, 2011) has shown that SGCs in TG are highly sensitive to ET-1, and we now asked whether this is the case for other peripheral ganglia. Application of ET-1 induced a clear rise in $[Ca^{2+}]_{in}$ in SGCs of NG (Fig. 1A). It can be seen in Fig. 1A that the responses do not display a plateau. The threshold concentration for evoking a response was 0.1 nM, and the concentration-response curve reached plateau at about 10 nM, with EC_{50} of about 1.4 nM (Fig. 1B). As a negative control we tested the responses to an unrelated peptide, bradykinin (20 nM), and observed no response. Responses to repeated applications of ET-1 showed considerable desensitization in the NG and Sup-CG, as reported for the TG (Feldman-Goriachnik and Hanani, 2011).

Next we tested whether SGCs in the mouse Sup-CG respond to ET-1. Application of ET-1 induced a clear rise in $[Ca^{2+}]_{in}$ in SGCs of this ganglion as well. The threshold concentration for evoking a response was 0.1 nM, and the concentration–response curve reached plateau at about 20 nM with EC_{50} of about 2 nM (Fig. 1C). The response time course was similar among the three ganglion types. On the average 95% of the SGCs responded to ET-1 in these ganglia.

3.2. Effect of extracellular Ca^{2+} on the responses to ET-1

To gain insight into the mechanisms by which ET-1 increased $[Ca^{2+}]_{in}$, we asked whether the responses to this peptide depend on the presence of extracellular Ca^{2+}. We measured responses to ET-1 (2 nM) in low Ca^{2+} solution (0.5 mM), and found that the responses of SGCs in NG and also in Sup-CG were reduced by about 42% compared with control - normal (2.5 mM) Ca^{2+} (Fig. 2). This indicates that the $[Ca^{2+}]_{in}$ elevation is at least partially due to Ca^{2+} influx from the extracellular space. Similar results were obtained for SGCs in the TG (Feldman-Goriachnik and Hanani, 2011).

3.3. Receptor types that mediate the Ca^{2+} responses of SGCs to ET-1

We used selective ETR agonists and antagonists to learn which ETR subtype mediates the responses to ET-1. In SGCs of NG the ET_BR agonist IRL-1620 (10 nM) evoked responses that were lower than to 10 nM ET-1. The selective ET_BR antagonist IRL-2500 (200 nM) reduced the response to ET-1 by 77% and the ET_AR antagonist BQ-123 reduced the responses to ET-1 by 85% (Fig. 3A). These results indicate a mixed ET_AR/ET_BR contribution.

Please cite this article as: Feldman-Goriachnik, R., Hanani, M., The effects of endothelin-1 on satellite glial cells in peripheral ganglia, Neuropeptides (2016), http://dx.doi.org/10.1016/j.npep.2017.03.002

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