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Research Report

Vasopressin-induced intracellular Ca^{2+} concentration responses in non-neuronal cells of the rat dorsal root ganglion

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ABSTRACT

Arginine-vasopressin (AVP) is a nonapeptide of hypothalamic origin that has been shown to exert many important cognitive and physiological functions in neurons and terminals of both the central and peripheral nervous system (CNS and PNS). Here we report for the first time that AVP induced an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in non-neuronal cells isolated from the rat dorsal root ganglion (DRG) and cultured in vitro. The ratiometric $[Ca^{2+}]_i$ measurements showed that AVP evoked $[Ca^{2+}]_i$ responses in the non-neuronal cells and these concentration-dependent (100 pM to 1 μM) responses increased with days in vitro in culture, reaching a maximum amplitude after 4–5 day. Immunostaining by anti-S-100 antibody revealed that more than 70% of S-100 positive cells were AVP-responsive, indicating that glial cells responded to AVP and increased their $[Ca^{2+}]_i$. The responses were inhibited by depletion of the intracellular Ca^{2+} stores or in the presence of inhibitors of phospholipase C, indicating a metabotropic response involving inositol trisphosphate, and were mediated by the V_1 subclass of AVP receptors, as evidenced by the use of the specific blockers for V_1 and OT receptors, $(d(CH_2)^1_5,Tyr(Me)^2,Arg^8)$-Vasopressin and $(d(CH_2)^1_5,Tyr(Me)^2,Thr^4,Orn^8,des\text{-}Gly\text{-}NH^9_2)$-Vasotocin, respectively. V_{1a} but not V_{1b} receptor mRNA was expressed sustainably through the culture period in cultured DRG cells. These results suggest that AVP modulates the activity of DRG glial cells via activation of V_{1a} receptor.

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1. Introduction

The effects of the nonapeptide arginine-vasopressin (AVP) on the central nervous system (CNS) where they originate from are well established and extensively documented. They range from control of water homeostasis to stress (Volpi et al., 2004), anxiety (Bielsky et al., 2004) and social behavioral modulations (Tobin et al., 2010). AVP is synthesized in and secreted by the magnocellular neurosecretory neurons of the hypothalamo-neurohypophysial system. The action of AVP is mediated through a specific subtype of 7 transmembrane domains G-protein coupled receptors. The AVP receptors are represented by 3 distinct subtypes classified as V_{1a}, V_{1b} and V_2 receptors (Manning et al., 2008). The AVP system is present and/or active in peripheral organs such as heart (Dayanithi et al., 2008), smooth muscles (Karashima, 1981; Li et al., 2001; Van Putten et al., 1994) and also in the peripheral nervous system (PNS) (Bone et al., 1984). In respect to the latter case, the actual physiological role that AVP could play is still under consideration.

Dorsal root ganglia (DRG) contain the cell bodies of pseudo-unipolar neurons conveying and integrating somatic sensory inputs (pain, temperature, mechanosensation) from the periphery to the spinal cord. According to somata's diameters, one can distinguish 3 types of neurons: large, medium and small, corresponding to different conductivities and sensory modalities (Scroggs and Fox, 1992). Obviously, the classification of DRG neurons is much more complex and is also based on the action potential configuration, the level of myelination and the expression of important molecular markers such as ion channels (Lechner et al., 2009).

DRG neurons are surrounded by both satellite glial cells (Hanani, 2005) and myelinating Schwann cells. Both types of the DRG glial cells are derived from neural crest (Jessen and Mirsky, 2005; Le Douarin et al., 1991), and express S-100 protein (Vega et al., 1991) but have several differences in their functions and localization.

The satellite glial cells surround completely around the cell bodies of DRG neurons, and the distance between the two types of cells is about 20 nm (Pannese, 1981). Little is known about the function of the satellite glial cells, but a recent study suggests that the satellite glial cell has a significant role in controlling the microenvironment in ganglia (Ohara et al., 2009). The satellite glial cells express the glutamate–aspartate transporter and glutamine synthetase (Miller et al., 2002) and may play an important role in maintaining glutamate homeostasis in the DRG (Bak et al., 2006). Another investigation implies the relevance between the satellite glial cells and the pain sensing mechanisms (Vit et al., 2008). Silencing of the inwardly rectifying potassium channels, which are expressed only in the satellite cells among DRG cells, results in appearance of both spontaneous and evoked pain and decreased tolerance to innocuous stimuli.

The major physiological role of Schwann cells is myelinating the axons of PNS neurons and thus facilitating the saltatory conduction. Schwann cells also contribute to the neuronal survival and nerve regeneration processes by providing neurotrophic factors (Madduri and Gander, 2010). After the denervation, Schwann cells produce many types of neurotrophic factors such as brain-derived neurotrophic factor, nerve growth factor (NGF), glial cell line-derived neurotrophic factor, which stimulate the axonal growth and support the neuronal survive (Shim and Ming, 2010).

Kai-Kai et al. (1986) have identified AVP immunoreactivity in neurons of rat DRG. Shortly after, a different group characterized the accumulation of inositol phosphates by application of AVP at concentrations in the micromolar range in rat DRG (Horn and Lightman, 1987), and this phenomenon was dependent on the V_1 AVP receptor subtype. Based on these studies, we could assume that: (i) AVP is also present in the PNS especially in neurons of the DRG and (ii) the inositol phosphate pathway is involved through receptors coupled to G_q proteins, suggesting the existence of AVP receptors in ganglia cells and the possibility for the peptides to induce the production of inositol 1,4,5-trisphosphate ($InsP_3$) responsible for the activation of Ca^{2+} release channels seated on the membrane of the endoplasmic reticulum. Within the huge family of 2nd messengers, Ca^{2+} is widely considered as the most ubiquitous and the control of Ca^{2+} homeostasis in cells is of major importance for the regulation of development, excitation, contraction, neurotransmitter release, aging and apoptosis (Thul et al., 2008). Taking all these aspects into consideration, the aim of the present study is to investigate the AVP-induced Ca^{2+} signaling in the cultured cells of the rat DRG. Our results show that AVP induces consistently a clear Ca^{2+} signal involving an $InsP_3$-evoked release of Ca^{2+} from the intracellular stores.

2. Results

2.1. AVP-induced $[Ca^{2+}]_i$ signals and immunoreactivity against S-100 in DRG cell culture

At six days in culture, various cell types were present in our DRG culture, that was distinguishable on morphological criteria (Fig. 1D). The first type was roundly shaped and had highly dense cytosol, resembling the morphology of the DRG neurons (Kitamura et al., 2005b) (a), while a second type was represented by polygonal or spindle shaped cells with lower density cytosol in phase contrast microscopy (b). To identify on a functional basis the types of cells, we have used a 60 mM KCl ($60K^+$) stimulation protocol (30 s) while performing $[Ca^{2+}]_i$ measurements. As illustrated in Fig. 1 A and B, showing an example of the $[Ca^{2+}]_i$ changes in a typical filed of the DRG culture, only one cell responded with a $[Ca^{2+}]_i$ increase to the $60K^+$-induced depolarization. Panel C in Fig. 1 shows the response of the culture to the bath application of 1 μM AVP for 1 min after the exposure to $60K^+$ for 30 s. The majority of the cells responded with a clear Ca^{2+} signal, while the neuronal cells failed to respond. This difference in response is more clearly illustrated by the $[Ca^{2+}]_i$ traces in panels F, where in a neuron (a) only $60K^+$ evoked a rapid sharp rise in $[Ca^{2+}]_i$, whereas in a non-neuronal cell (b), only AVP evoked a long-lasting $[Ca^{2+}]_i$ increase.

In the DRG, there are two types of glial cells, the satellite glial cells and Schwann cells, and both show the expression of the S-100 protein (Vega et al., 1991). Thus, to confirm the glial nature of the cells responding to AVP but not to $60K^+$